

US chauvinism and electronics

A report from the Office of Technology Assessment should help to correct the widespread underestimation in the United States of the strength of its electronics industry.

THE most remarkable feature of the report of the US congressional Office of Technology Assessment (see page 305) is that it was ever considered necessary. Briefly, the document concludes that the US electronics industry is in good shape, is technically capable of keeping up with that of Japan but is likely to encounter increasing competition in the foreseeable future. In particular, the Office of Technology Assessment solemnly says that reports from Japan of the deliberate development of an ambitious "fifth-generation computer" (in a lineage whose previous generations are undefinable), supposedly masterminded by the Ministry of International Trade and Industry (MITI), is not the threat it has been made out to be. To most people, these conclusions will seem platitudinous. But in the United States, where the invention of external bogey-men seems an unavoidable part of public discussion (and not merely of technology), the new report (if it is read at all) will be more of a surprise.

The supposed threat from Japan is an illusion much in need of exorcising. It has become a kind of collective nightmare, the other side of the coin from the American dream. It stems from a profound misunderstanding, apparently made worse by the endless scholarly studies by US academics in the past fifteen years of how Japanese industry functions. The popular legend, apparently invented so as to account for the commercial success of Japanese companies in the export trade, is that government ministries in Tokyo cleverly identify new export markets, cunningly organize Japanese companies for their exploitation and then back the whole enterprise with the necessary funds for research and development.

This is a travesty of reality. The much-feared fifth-generation computer project consists of little more than a series of committees (set up, admittedly, by MITI) to look into such questions as the feasibility of translating the sounds of voices into computer code. The total cost over five years of the research and development falling within the scope of the project (most of which will be borne by Japanese companies) is less than the budget for the programme of research and development in information technology sponsored by the British Government along similar lines. Moreover, as the most casual acquaintance with Japanese companies should make plain, the shots are being called not by the ministry but by the companies. And they are understandably more immediately concerned with their domestic market (where they enjoy the huge benefits of largely chauvinistic purchasers as well as the protection of a series of unfair trade practices) than with exports. The fact that Japanese companies are generally superb at the organization of manufacturing production may eventually allow some of them to compete even with the successful US computer manufacturers in sophisticated hardware. But that is not nearly as much of a threat as the common paranoid appraisal of it in the United States implies.

The US Administration needs a better understanding of the true position not merely for the sake of relations with Japan but so that it can handle more confidently, and less dangerously, its policy on what is called the "export" of technology (see page 305). What the United States forgets is not merely that its own electronics industry is at present strong but that its spending on research and development is by any standards enormous. And while some of these resources may be wasted, as when they lead to the production of yet another personal computer by a corporation

that promptly goes out of business, the scale of spending by the IBMs and AT&Ts of this world is a telling pointer to what probably lies ahead.

In computing machinery, for example, the chances are that products now on the market will be recognized only a few years from now to be primitive machines. And while there may be some benefits in tricks such as the conversion of human voices into computer code, developments will come from innovations of computer architecture, and from the design of computer software, that will give these plodding electronic structures a greater capacity to simulate thinking than they have at present. By the end of the century, machines such as those on the way to the Soviet Union in the past week in West Germany and Sweden will probably be lumped together in the history books with Babbage's computing engine. Commercially, the freedom that AT&T will acquire on 1 January to manufacture and sell computer hardware will be an important and constructive landmark, one likely to give the lie to last week's opinion from the technology assessors that the break-up of the telephone company will put a national resource in hazard.

From this perspective, much of the US Administration's attitude towards high technology is misconceived. To be sure, it is sensible enough that the anti-trust legislation should be clarified so as to make it plain that companies may collaborate with each other on basic research without falling foul of legal proceedings. The strategic embargo on the export of advanced equipment is similarly sensible, but only because of the parlous condition of relations between the Soviet Union and the United States; commercial competitors elsewhere would gain little from making slavish copies of today's technology. And the constant anxiety that open discussion within the technical community may allow others to steal a march on the United States is a foolish oversimplification, demeaning to the people and the companies in this line of business, who have hitherto succeeded well enough in talking about what they have done while doing what others fail to accomplish. To cramp that process, one of the chief means by which small companies in the United States acquire skills from their larger competitors as well as from companies elsewhere (Japanese speakers are now conspicuous at electronics conferences in the United States) could be like shooting oneself in the foot, as must be any interference with the customary flow of scientific ideas. □

Research in trouble

Britain's chief instrument for supporting university research faces tough choices.

DURING the past five years, the British research establishment has done reasonably well, no doubt because the Prime Minister, Mrs Margaret Thatcher, promised at the outset of her government that what is called the science budget would be "protected". At least on paper, the promise will be kept for next year. The total science budget will even increase by 6 per cent, faster than inflation, and there is no serious cause to fear that circumstances will drastically change in the years thereafter. So why are the research councils, the chief dependants of the science budget, and the chief source of support for independent research in Britain,



likely to be downcast by last week's apparently good news?

The annual report of the Science and Engineering Research Council for the year ending in March, published this week, is not much help towards an explanation. The council, the largest of the five operating in Britain, spent £244 million in the year concerned, almost exactly a quarter of it on research grants to people working in universities. In cash terms, the council's spending increased by roughly ten per cent in comparison with the previous year (roughly comparable with the then average rate of inflation and thus not worth much). Like a good but cautious housekeeper, the council seems to have distributed its resources uniformly across the board. Indeed, contrary to expectation (and popular legends sometimes current), the council's own research establishments were less generously dealt with than if there had been a uniform distribution of resources. Between the years in question, even the cost of international subscriptions (to places such as the European Organization for Nuclear Research (CERN) at Geneva) did not increase significantly faster than inflation (but will next year cost the council an extra £10 million).

Three problems afflict the council, one of which is that it has been obliged by pressure from without to devote substantial if still marginal sums to fashionable causes, research in engineering or information technology, for example. This means there is less to spend on other things. Second, the council is increasingly the prisoner of circumstances only formally within its control. For while the council exists only for the support of basic research in universities, it managed last year to spend directly on these purposes only 56 per cent of its total budget (£69 million on research grants, £35 million on support for graduate students). The rest of the budget went on administration (a mere 3 per cent) and on the provision of central services for the use of researchers in higher education. Nobody complains that these central services were badly managed. The question that needs asking is whether, in present circumstances, this distribution of the council's resources is that most appropriate to its central purposes.

The simple answer is that it cannot be. For the most serious of the problems facing this research council (as the others) is the virtual collapse of the dual-support system, the fiction once fact that universities would devote a substantial part of their general income to provision for research by their academics. The consequence is that, inflation apart, a research grant now is worth less than what the same grant (in real terms) would have been only a few years ago. Some of it will have to be spent on basic equipment that in the old days the universities would have provided. Because there is no prospect of change for at least the next three years, and since there is no reason to suppose that the largest of the five research councils, and the only one among them with terms of reference requiring the support of university research, is careless of its objectives, it has a painful choice ahead of it: which facilities should be closed down, or which international subscriptions should be forgone, to make room for a sufficient support of basic research? The council was edged earlier this year in that direction by the report of the government's efficiency audit, which recommended the merging of two observatories on managerial grounds. In the new circumstances, this process will have to go further and faster than the council was planning only a few months ago, especially because the council's expected budget is likely to be raided to help with the cost of reorganizing internal institutes managed by other research councils. The extent of that will not be known for a month.

Other research councils will have more serious trouble. Those responsible for agriculture and the natural environment know something of what lies ahead. The Medical Research Council is likely also to have its wings clipped next year to help reorganization elsewhere. Although this council's terms of reference are different, it managed last year to increase its general research grant spending (about a quarter of the total) by a mere 1 per cent in cash terms. Its problem is its commitment to its own people and institutions. That, too, may have to be reduced. Indeed, the medical council seems to have made a start last year by cutting back at its expensive Clinical Research Centre. Worse, no doubt, will follow. □

Europe in bottles

The common market should be made to function in spite of British defence of bottled milk.

THE meeting of the European Community arranged for Athens early next month is being advertised by the governments concerned as crucial. The immediate problem is that the Community has run out of funds. But decisions must also be made about the whole basis of collaboration within the Community, perhaps even about its continued existence. The agenda for Athens includes the reform of the Community's agricultural policy, the device for supporting over-production by overcharging Europeans for the food they eat, but the European Commission's attempts to devise a compromise between the beneficiaries and the victims of this arrangement seem so far to have failed. The pressure for some kind of agreement has been increased by the insistence of Britain and West Germany that the future of sensible research programmes such as the planned collaboration in information technology should be left for decision at Athens (see *Nature* 10 November, p.103), while both the Commission and some member governments are saying that Athens should be an opportunity for making the Community function as a common market, the central provision of the Treaty of Rome now widely breached by the bureaucratic restraints on trade that governments increasingly substitute for tariffs.

The outlook is not bright, as can be told from last week's debate in the House of Commons about the British Government's reluctant decision to allow the import from elsewhere in the European Community of milk (called UHT) that has been treated at 120°C for two seconds, which as a consequence lasts for a long time but which is less palatable than fresh milk. The British Government has had no choice. It was found guilty by the European Court last February of operating an illegal restraint on intra-European trade — the simple expedient of insisting that imported milk should be heat-treated a second time. The new regulations, debated last week, are the British Government's compliance with the court's judgement.

Legality seems to count for little. Politicians of all stripes last week joined in protest at the prospect that horrid mainland milk would find its way to previously uncontaminated Britain. The government's defence was about as lame as it could have been. Mr Michael Jopling, the agriculture minister, relied chiefly on the claim that even the new regulations on milk imports to Britain will be an encumbrance to would-be exporters — milk imports will be allowed at only 17 of 77 possible ports of entry, each consignment will have to wait until it is inspected, special restrictions will be applied when foot-and-mouth disease crops up in exporting countries and so on. For his pains, he earned nothing but the contumely of those Members of Parliament who plainly would have welcomed a much more devious strategy, perhaps along the lines of the now-famous French device for keeping out Japanese video-recorders by sending them for inspection to an overcrowded warehouse at Poitiers. The device of requiring that a sample of each consignment of imported milk should be kept on the shelf for its advertised lifetime before the rest of it could be sold would obviously have done the trick.

The offensiveness of this position lies in its hypocrisy. The British Government seems to be telling the world at large that it wants to make the European market efficient, but then telling the House of Commons that "we must comply with the judgement, but that does not mean that there has to be a free-for-all". In passing, the government joined with its critics last week in promising to defend with its whole power the curiously idiosyncratic British institution called "doorstep delivery" by means of which most British milk is sold in glass bottles which are left at intervals along public streets in such a way as to assist bacterial culture and add perhaps 20 per cent to the cost of milk. At no stage did the government choose to make palatable its compliance with the law by insisting on people's proper expectations that milk will be sold as competitively as possible. In such circumstances, it is no wonder that hopes for the Athens meeting are only meagre. □

US research restrictions

Policy on high-tech data threatens freedom

Washington

THE United States is stepping up its efforts to prevent American high technology from falling into the hands of the Soviet Union or other potential adversaries. At the instigation of the US Department of Commerce, West German authorities last week seized a VAX 11/782 computer minutes before it was to leave Bonn for the Soviet Union. Another was seized in Sweden at the week-end. Earlier in the month, Federal Bureau of Investigation (FBI) agents infiltrated a meeting in Boston of the American Vacuum Society and arrested Alfred Zehe, an East German physicist suspected of espionage. And in revised regulations for Soviet diplomats and journalists, the State Department has declared "Silicon Valley", near San Francisco, out of bounds.

The administration's mounting offensive against the leakage of high technology continues to worry many scientists. A special report published last year by the National Academy of Sciences under the chairmanship of Dale Corson, former president of Cornell University, conceded that Soviet military power had benefited from the acquisition of Western high technology. But it said that scientific communication accounted for only "a very small part of this transfer" and warned that measures to control technology transfer could — if pushed to extremes — have a chilling effect on American science.

Since the Corson report was published, there have been few signs that the administration has succeeded in bringing order to the patchwork of laws and regulations that govern technology transfer. At recent congressional hearings, Dr Frank Press, president of the National Academy of Sciences, expressed alarm at new government proposals to require scientists to secure government permission before they make unclassified research results available in foreign countries — a measure that would affect virtually all scientific publications since almost all have an international readership.

What was most disquieting, Dr Press said, was the impact on individual scientists. Government action to control scientific communication had been largely disjointed, unpredictable and vague. "The result is that any particular scientist is quite unclear about what obligations and sanctions, if any, might apply to his or her work."

The present system of controls involves about 44 agencies in 10 government departments — a recipe for confusion and inconsistency. But there have been some signs of improvement. Two recom-

mendations of the Corson report have been implemented: the intelligence community has created a scientific advisory panel to comment on prospective scientific exchange visitors from adversary nations and the National Academy of Sciences has established a government-university round table to resolve conflicts between the government and the research community.

In Congress, too, pressure from the scientific community has influenced debate on the future of the Export Administration Act which expired in September. Both the House and the Senate version of the successor act contain a two-sentence paragraph stating that it is the policy of the United States to sustain a vigorous scientific enterprise and that to do so requires that scientists and scholars be allowed to communicate freely.

But none of these initiatives, Dr Press complained, tackles the major provisions of the Corson report. After reviewing much classified information about technology leakage, the Corson panel suggested that most university research could be freed from controls. Controls could be applied to the remaining "grey areas" only if specific criteria were met. For example, the technology would have to

be capable of giving the Soviet Union a significant near-term military benefit, and the United States would have to be the only source of information about the technology.

The administration's apparent inaction in the aftermath of the report appears to be a result of a change in the structure and terms of reference of the groups set up to respond to it. After publication of the report in November last year, the White House Office of Science and Technology Policy was asked to produce a response by March. In February, however, the study panel became part of a wider study of technology transfer under the direction of the National Security Council. The new group is expected to report next month, but there is a strong possibility that its conclusions will be classified.

In his evidence to Congress, Dr Press said he was disappointed by the delays and by the failure of the National Security Council to seek the advice and help of the outside scientific community. He warned that excessively harsh restrictions on scientific communication were likely to be counter-productive. Restrictions on foreign scientists at scientific meetings could force international scientific organizations to hold their meetings outside the United States. And, more important, there was the additional danger that burdensome government restrictions would tend to drive talented scientists out of restricted research areas, depriving American military technology of their contributions.

Peter David

National Institutes of Health

One set-back for Waxman bill

Washington

THE bill that would have written into law specific research directives for the National Institutes of Health (NIH), created a new arthritis institute and set up a commission to monitor human genetic engineering faltered last week despite a last minute compromise between its supporters and opponents in the House of Representatives.

The compromise, which ended a year-long impasse between Representative Henry Waxman (Democrat, California) and Representative Edward Madigan (Republican, Illinois), removed the provisions that NIH had found most offensive. Waxman had wanted to change NIH's research authority from the broad discretionary one it now enjoys to a line-by-line authorization for specific research areas. Madigan — and NIH — were pressing hard to retain NIH's freedom to set its own research priorities on the basis of scientific advice rather than legislative fiat. The compromise deleted the line item authorizations, while retaining general instructions to NIH to conduct certain specific research programmes. It also removed a controversial proposal which would have transferred the National

Institute of Occupational Safety and Health to NIH.

The bill passed the House the day before Congress adjourned until January. In the Senate, however, a deadlock over fetal research prevented even consideration of the bill. Senator Bob Packwood, a liberal Republican from Oregon, refused to allow either the House version or a version provisionally accepted by a Senate committee from reaching the floor so long as conservative Senator Jeremiah Denton (Republican, Alabama) threatened to introduce an amendment banning the use of NIH funds for research involving fetuses. (The House performed a *volte face* on the issue, first adopting a similar ban offered as a floor amendment, then negating it by passing another amendment.)

NIH have standing authority under the Public Health Service Act for most of their activities, so the net effect of Congress's inaction on the bill is small. Funds for assistance to medical libraries and for training grants were however shunted into the continuing resolution as a result, and were thus held to fiscal 1983 levels.

Stephen Budiansky

British science budget

More money will buy less

NEXT year's budget for British science is a disappointment, not least for the research councils. Planned provision for education and science in 1984-85, announced in the House of Commons last week by Sir Keith Joseph, Secretary of State for Education and Science, will increase by 4 per cent in cash terms, less than the inflation rate.

The science budget, out of which the five research councils receive most of their income, will be £549 million, 6 per cent more than in this financial year and £6.5 million more than was foreseen in the government's March expenditure white paper. But almost all the extra is earmarked to meet the cost of international subscriptions, which chiefly affects the Science and Engineering Research Council (SERC). Otherwise the budget remains roughly constant in real terms.

The recurrent grant to the universities channelled through the University Grants Committee is planned to be £1,265 million for the academic year 1984-85, a cash increase of 4.4 per cent over the current academic year.

The science budget will be divided between the research councils on the basis of recommendations by the Advisory Board for the Research Councils (ABRC) next month, when it will publish suggested allocations for 1984-85 and the two following years. But it is clear that Sir David Phillips, the council's chairman, has been largely unsuccessful in his bid for extra funds to pay for restructuring programmes planned for the Agricultural and Food Research Council (AFRC) and the Natural Environment Research Council (NERC).

ABRC had asked for £35 million above the £543 million originally planned for next year to allow for "the continuation and development of existing policies". Sir Keith was apparently unimpressed: apart from the £6 million offered for international subscriptions, his response was a paltry £0.5 million, to cover restructuring costs.

The changes planned for the Agricultural and Food Research Council, for example, will require about £5 million next year and twice that amount in the two following years. In 1985-86, there may be some shifting of this burden onto SERC and the Medical Research Council, but for next year at least, ABRC plans do not envisage any major shift of funds towards AFRC and NERC. The inescapable consequence is that both will have to indulge in some drastic pruning in their own institutes in order to meet commitments to maintain university-based research.

The Treasury, as expected, has accepted the principle that Britain must collaborate with other nations in "big science" projects. The question of how SERC is to meet the spiralling cost of subscriptions to organizations such as the European

Organization for Nuclear Research (CERN) and the European Space Agency (ESA) has been the subject of a special review by a departmental committee under the chairmanship of Miss J. Kelley, an Under-Secretary at the Treasury. While recognizing the Kelley recommendation as a small victory, some SERC council members are worried that the amount provided will still be inadequate. The value of sterling has fallen over the past year, while the cost of subscriptions based on gross domestic product has increased. As a result, current estimates are that SERC needs an extra £10 million.

The argument within SERC on the balance between "big" and "little" science will certainly be fuelled by the recognition that the council therefore appears likely to

face a cut in real terms of something like 2 per cent. It is already known that ABRC bids for funds for a British-led space programme and for more university grants have fallen on stony ground. As SERC's financial position was already precarious, some members now fear that SERC laboratories will have to close and staff be made redundant so as to pay for closures in the institutes of other research councils.

The universities' recurrent grant is a small reduction, in real terms, from the present year's total. This comparison, however, disguises the fact that this year's total was reduced by £24 million in July, because university restructuring was costing less than had been expected. Mr John Akker, general secretary of the Association of University Teachers, said last week that he was nevertheless relieved that the total will be enough so that compulsory redundancies should be avoidable in the year ahead.

Tim Beardsley

Testing laboratories

UK presses for agreement

"MADE in Britain" will once again be something to boast about if new government initiatives to be announced this week succeed. A major publicity campaign will be launched to persuade industrial companies to make more and better use of recognized engineering standards and quality assurance procedures in product design and manufacture.

The start of the campaign coincides with the government's response to a report published last year* by the Advisory Council for Applied Research and Development (ACARD) on the impact of technical standards and regulations on product design and international competition. ACARD found that the existing schemes of the British Standards Institution are patchily developed and under-used. Conformity is often claimed fraudulently, and British standards are not widely accepted overseas. Unlike most industrial countries, Britain has few government-supported certification and approval schemes (those that exist are mostly concerned with safety) and industry is consequently unused to operating within such frameworks.

ACARD concluded that, despite the provisions of the General Agreement on Tariffs and Trade and the Treaty of Rome, some foreign governments were at least acquiescing in allowing technical standards to be deployed as obstacles to free trade. ACARD urged that industry and government should introduce more certification and approval schemes, particularly for internationally tradeable products. It advised the government to establish a national accreditation scheme for certification and approval bodies, to operate some controlled national mark for quality. Most of ACARD's recommendations will be accepted and implemented by the

government.

The new initiative will try to convince industrial companies that exports can be improved by demonstrating conformity with local approval criteria. There is widespread ignorance and disregard of such guidelines in Britain, and the technical help for exporters offered by the British Standards Institution is over-priced and under-used. Smaller companies, in particular, often fail to take into account the technical requirements of overseas countries at a sufficiently early stage in the development of a product, so that expensive redesigning becomes necessary.

When a product does satisfy certification or approval requirements, manufacturers complain that overseas customers will not accept British laboratory reports, so tests have to be repeated — often at considerable expense — abroad. These complaints were the spur to a scheme which now accredits testing laboratories in Britain with a view to establishing wider recognition of their reports.

The International Laboratory Accreditation Conference (ILAC) has for a number of years been working towards greater international acceptance of test results, and at its meeting in Prague earlier this month the conference was told that a memorandum of understanding had been signed between the US National Bureau of Standards and the British National Physical Laboratory, providing for mutual recognition of their respective test laboratory accreditation schemes.

The British scheme, NATLAS, has been in operation for only two years but already covers more than 200 laboratories in the fields of mechanical, electrical, physical and chemical testing. In the United States, the equivalent scheme is the National Voluntary Accreditation Program

(NVLAP), which despite the plethora of certifying bodies is slowly becoming accepted throughout the country. Under the recent agreement, laboratories covered by the two schemes will recognize each other's reports and will recommend third parties to do likewise. Calibration certificates for measuring instruments are excluded from the memorandum, but may become recognized for certain quantities in which national measurement standards are deemed to be equivalent to specified accuracy.

Although nobody pretends that trade between Britain and the United States

will be greatly affected by the NATLAS/NVLAP agreement, the deal is seen as a significant pointer to future developments: Britain is already actively negotiating similar understandings with Australia and New Zealand. But many British manufacturers will really become interested only when comparable agreements are reached with West Germany, and, especially, France, which is held by many to have been particularly obstructive in the past.

Tim Beardsley

**Facing International Competition: The Impact on Product Design of Standards, Regulations, Certification and Approvals. ACARD, July 1982, HMSO.*

West German biotechnology

Labs fight shotgun marriage

A DISPUTE is simmering in West Germany over the recommendations on state-financed biotechnology made to the Federal Ministry of Research and Technology (BMFT) by a specially appointed commission. Two institutions are principally involved: the Gesellschaft für Biotechnologische Forschung (GBF) at Braunschweig-Stockheim and the Institut für Biotechnologie (IBT), part of the Jülich nuclear research establishment east of Cologne.

GBF evolved from a molecular biology research institute founded with the help of the Volkswagen Foundation in the 1960s to provide research materials for universities and Max-Planck Institutes. Since 1976, it has been financed 90 per cent by the federal government and 10 per cent by Lower Saxony, conducting research in biosynthesis, animal and plant cell culture and enzymology, but also being actively involved in education and process development. It is the home of the German collection of microorganisms and has a staff of 350 scientists, 10 departments and a budget of DM27 million (£7 million) a year.

IBT at Jülich is smaller, with a staff of 100 in three departments: microbiology, chemical engineering and algae. Its main focus is on enzymatic polysaccharide degradation, biocatalysis and biological water purification.

The commission was set up by BMFT, in part in recognition that West Germany lags behind the United States in this economically important area. BMFT has meanwhile decided to increase investment in biotechnology from DM63 million in 1983 to DM70 million in 1984. The commission's chief recommendation is that the two institutes should be amalgamated and that research support be orientated towards "special project" financing, on the grounds that it would be more flexible than institutional support.

Despite several important exceptions, the report criticizes the patchy standard of research at GBF. It says there is a lack of internal collaboration and recommends that GBF should be reformed into four departments: process development, enzyme

technology, microbiology and cell biology, and that plant cell culture and cellulose degradation should be dropped, as should the algal research at IBT.

The report also says there is "no meaningful future for GBF" without a radical reorganization designed to raise the scientific competence of the supervisory board, including more external members, and unless its part in the management, control of quality and direction of research and staffing is redefined and strengthened. The report goes on to say that the "healthy cores" of GBF and IBT should be concentrated in one institute at Braunschweig or, as a second best, that some GBF departments should be moved to Jülich, leaving a group of industrially-orientated projects, akin to those of a Fraunhofer organization, at Braunschweig.

Three-way negotiations (with BMFT) are going on, but first reactions at both institutes have been outspokenly negative. Each complains that the commission failed to publish details of the scientific assessments behind its conclusions. GBF points out that its total budget is equal merely to the electricity bill at the DESY high-energy physics laboratory at Hamburg and that, unlike the other eleven large research institutes, it has an income of DM1.3 million from licences and research. It also argues that the commercial potential of projects at GBF have been underestimated.

The departments at Jülich say that they would be used to revamp GBF in a way that jeopardizes their incontestable effectiveness and success and would destroy valuable academic and industrial links. They also feel that the research on algae which is aimed at water purification has been undervalued.

According to a BMFT estimate, total investment in biotechnology in West Germany is running at an annual DM300-500 million, some four-fifths of it in industry. A particular complaint at the two institutes is that a commission comprised 50 per cent of industrial representatives should advocate increased support for the "special project" system.

Sarah Tooze

Soviet spaceflight

Evacuation of Salyut begins

SOVIET cosmonauts Vladimir Lyakhov and Aleksandr Aleksandrov will be preparing to return to Earth "within a few days", Moscow radio announced last weekend. Mothballing of the Salyut station began on Sunday, and experimental material had been transferred to the Soyuz-T9 transporter.

This apparent commitment to bring the cosmonauts back aboard the Soyuz-T9 has confounded Baikonur-watchers who, for the past two months, have been predicting disaster, because the Soyuz-T9 has overrun by half as much again the 115-day safe working life accepted by the Soviet space planners. Since a replacement Soyuz craft blew up on the launch-pad on 26 September, there has been considerable alarmist speculation about how the two cosmonauts would return. But Mr Geoffrey Perry, of the group at Kettering School in England which has been monitoring the cosmonauts' conversations, has maintained throughout that the cosmonauts were never in any jeopardy, that their conversations with ground control have consistently reported everything normal and that his group had no evidence for or against the fuel leak of 7 September reported by *Aviation Week*.

The implication is that the 115-day safe working life of the older Soyuz model is no longer relevant for the improved Soyuz-T. In contrast with the earlier Soyuz, which used hydrogen peroxide as a separate propellant for the reaction control system, the Soyuz-T uses the same fuel system (hydrazine plus a nitrogen oxide, tetroxide) for both the main propulsion system and the control thrusters. This eliminates a major factor in calculating the lifetime of the on-board consumables — the long-term storage of hydrogen peroxide. It also gives a high degree of manoeuvrability, "especially in unforeseen circumstances" according to Soviet sources.

The outstanding problem now is to identify the source of the rumours of impending disaster which have been prominent in the Western press during the past two months. *Aviation Week*, although the source of the reported fuel leak, now seems to have tacitly disclaimed the scare by noting in its most recent issue that flight controller Aleksei Eliseev is optimistic about a successful return. Unfounded disaster reports have bedevilled the Soviet space programme for more than twenty years, and seem an unavoidable spin-off from the secrecy with which that programme has always been surrounded. It is ironic, just when a certain degree of openness about Soviet space plans and facilities is becoming apparent, that yet another such rumour should receive so much attention in the West.

Vera Rich

Research in France

All fonctionnaires now

FROM the new year, all 12,000 French research council researchers and the 28,000 engineers (senior technicians), technicians and administrators who work with them will become civil servants, enjoying the security and pension rights dreamed of by every French man and woman. But fears that the 40,000 people concerned will now be bureaucratized, like the civil-servant scientists of Italy, are denied. According to Pierre Papon, director-general of the Centre National de la Recherche Scientifique (CNRS), on Monday, the move should "decrease the viscosity" of the French research system and be "a tool of science policy".

The new statute defining the admission and promotion of scientists and other ranks has been through 50 hours of round-table negotiations and consultations and has emerged riddled with "dérégulations" — special exceptions that will distinguish it from the normal law of the civil service. For example, unlike the French civil service which can normally be entered only from the bottom grade, the new "research service" will have openings at every level, allowing senior scientists to be brought in from universities, industry or abroad and adding a horizontal dimension to the usual civil service verticality.

Such exceptions make the statute workable — but at the same time, the statute offers researchers lifetime employment from entry, at the age of 26–27, after completion of a thesis. This, the argument goes, should make for greater mobility as

people will have no fear of losing their jobs. Posts will also be advertised on the open market, giving Papon and colleagues such as Philippe Lazar, director-general of the medical research council INSERM, "the chance to match science policy to the distribution of posts".

However, Papon and Lazar were not always so happy. In the summer, they were said to be thinking of abandoning the whole project because the statute then under consideration was too rigid. The chief difficulty was that ministers who manage large numbers of civil servants feared that their employees might also demand the same terms — such as freedom to create businesses (included in the research statute) or to be promoted between "corps" (categories of employment) on the basis of professional experience (normally, only paper qualifications count).

In the end, however, the Minister of Research and Industry, Laurent Fabius, won the day. Papon says "the text is not too difficult. It is 'in phase' with CNRS."

According to Claude Kordon, a director of research with INSERM who was the chairman of an independent commission on the research statute, the result is "a symbolic breakthrough". Other elements of the civil service — such as the universities, which await a similar statute defining their responsibilities — may be forced to follow suit, particularly in adopting a regular assessment of performance. (This is implied by relating promotion and advancement to professional experience.) At pre-

sent, advancement in universities is less dependent on performance than on seniority, Kordon believes.

Some of the main points in the new statute are:

- Technicians may be promoted on the basis of work, not age; previously only age counted, and a survey last year at CNRS showed that up to half of CNRS technicians were under-graded as a result.

- Apart from job security, there are positive incentives to mobility, such as an extra salary step for a change of job and the consideration of breadth of experience in the appointment of directors of research. It will be possible to move into industry for a few years, for example, and then to return to one's job, to another research institution, to a university or to go abroad.

- The objective of a research career is broadened, to include training (of research students, for example), popularization and application of results (such as the deposition of patents). These will be taken into account during the interviews and publication reviews that lead to promotion.

- A very successful person can change corps — for example from technician to engineer, or secretary to administrative grade.

The new statute has some defects, however. Kordon emphasizes his use of the word "symbolic" and says that it may take technicians 12 years and scientists 6–10 years before they come to their first evaluation, so that the incentive is not high. Moreover, the commissions that will determine an individual's salary increments will include no scientists, only union and administrative people. "The coupling between a career and evaluation is too loose", says Kordon.

Indeed, the idea of accelerated promotion based on performance could falter for lack of vacancies at higher levels. Most French researchers were recruited in the late 1960s and are now middle-aged and middle-ranking.

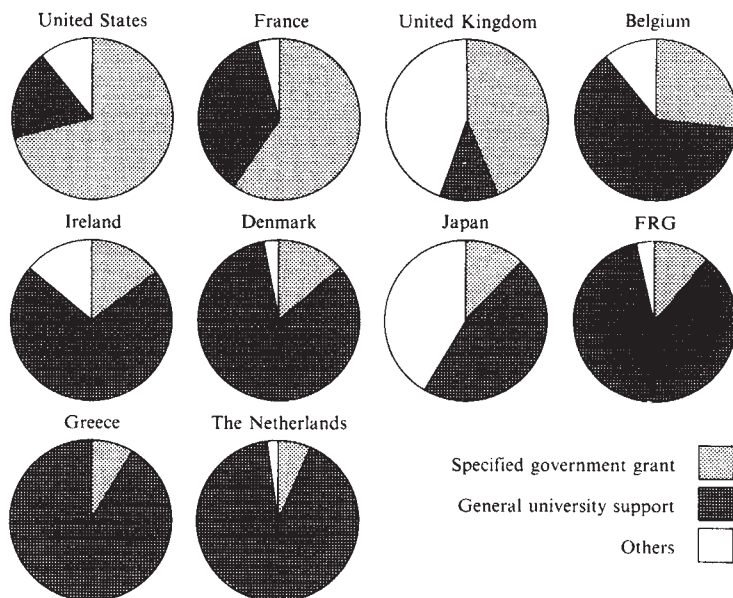
Kordon and his team recommended to the minister that he should adopt a "promotion policy" that would aim to smooth the demographic structure over a period of, say, 10 years, but this was rejected.

Papon is also concerned about the detailed application of the statute — which will be defined by separate detailed decree for each organization.

Exactly how to assess the performance of technicians and administrators, who outnumber scientists 2:1 and who change jobs more frequently, remains to be determined. The assessment will have to be local and often rather than national and annual, as for researchers, Papon believes. These and other difficulties have led to a 50-page draft decree for CNRS which will supplement the 250 articles of the statute itself. The decree could not be ready before March or April, Papon thinks, and will then have to be applied retrospectively.

Robert Walgate

Research spending in OECD countries



Specified government grant is defined as the money going to universities that is earmarked for research (such as that from the British research councils). General university support is an estimate sent in by each OECD country of how much of the total government grant is used for research and development. "Other" funds include those from industry, higher education itself and from other countries.

US electronics industry

OTA discounts threat of Japan

Washington

THE United States holds a virtually unassailable lead in the computer industry and is still well ahead of the rest of the world in semiconductor technology. That is the unfashionable conclusion of an important new study of the world electronics industry produced by Congress's scientific think-tank, the Office of Technology Assessment (OTA).

While it acknowledges the virtual collapse of the US consumer electronics industry — no video cassette recorders are made in the United States — the report says the American lead in marketing data processing systems is so large that prospective competitors, including those in Japan, can hope for only "modest" success over the rest of the century. An American company, IBM-Japan, is by far Japan's biggest exporter of computers and Japanese manufacturers of commodity-like chips, notably random access memories (RAMs), lag behind in the production of other microelectronic devices such as logic chips or microprocessor families.

The OTA study also pours cold water on the complaint that American companies have been fatally hampered by the centrally coordinated industrial strategies pursued by foreign trading rivals. The United States, says the report, could learn much by studying foreign initiatives such as West Germany's Fraunhofer Gesellschaft or Japan's government-supported VLSI (very large scale integration) projects. But government-led industrial policies are not enough to give any country a major advantage; the really important factor is the capability of a country's private companies and its technological ability.

In the case of Japan, the report concludes that the highly publicized VLSI project so envied by American companies entailed less cooperation between rival firms than is commonly believed. It was effective because it accurately targeted specific weaknesses within the Japanese electronics industry, such as its dependence on imported semiconductor manufacturing equipment. OTA believes that this kind of close attention to specific industrial problems, together with an emphasis on the long term, is what distinguishes Japan from its competitors.

Although OTA emphasizes the role of private companies, it does not dismiss the contribution a federally orchestrated industrial policy could make to the competitiveness of the American electronics industry. Public policies, the report says, set many of the "rules of the game" within which private enterprises compete. At present, some of the rules bind American companies more than is necessary.

One example offered in the report is the chilling effect of antitrust legislation. The break up of American Telephone and

Telegraph (AT&T) will, OTA claims, "inevitably" weaken the foundation of basic electronics research laid by the Bell Laboratories. Unless Bell's place is taken by some new mechanism for performing basic research and spreading its results, "one of the great sources of strength of the entire US electronics industry will atrophy".

The report applauds some of the current measures through which the government is increasing electronics research — through the Department of Defense's VHSIC (very high speed integrated circuit) programme and the \$20 million a year being spent on the development of gallium arsenide devices by the Defense Advanced Research

Projects Agency. But it says the general level of research underpinning the industry is inadequate, and questions the value of two much-publicized ventures — Admiral Robert Inman's Microelectronics and Computer Technology Corporation (MCC) and the Semiconductor Research Cooperative (SRC).

Both SRC, an industry cooperative organized to support university projects, and MCC, an independent profit-seeking venture, are designed to develop technologies that will benefit a number of companies. The intention is to avoid duplication, help disseminate research findings and undertake projects that will take longer than individual companies could contemplate. According to OTA, however, it is far from clear that either venture will fill the growing research vacuum.

Peter David

Electronic publishing

European joint development plan

Two European electronic journals, published entirely without the intervention of paper, will be a reality next year. The journals, one in information science and the other in computing, will be launched as part of an ambitious European Communities (EEC) experiment in electronic document delivery called "DOCDEL".

European Commission officials claim that the experiment will give Europe a world lead in the technology, administration and legal problems of electronic publishing. One objective is to set standards (formats, transmission codes, etc.) that others may later have to follow.

DOCDEL was unanimously approved last week by a committee of government representatives and seems certain to jump the final hurdle of the European Council of Ministers in a few weeks' time. The first ten experiments in the DOCDEL programme will cost £9.2 million, of which the Commission will pay only £2 million. The rest is covered by the contracting partners in the experiments.

One of the chief components of the new programme is "TRANSDOC", a comprehensive French experiment in scientific publishing. The bodies involved in TRANSDOC include the Centre de Documentation Scientifique et Technique (CDST) of the research council, the Centre National de la Recherche Scientifique (CNRS); the French patents offices (INPI); and the national electricity utility (EDF). Between them these organizations file some 600,000 pages of articles a year, all of which might ultimately be made available electronically — by agreement with the original publisher, to whom copyright fees will be paid. Experimental users of TRANSDOC will be the European patents office in The Hague, EDF, a group of biochemists in the Rhône-Alps region and visitors to the medical library of the University of Louvain. The experiment will be

reviewed at the end of 1985 and should by then have involved storage and accessing of documents on optical digital disc, on magnetic disc and on automatically accessible microfiche as well as transmission and reception by every available means, from mere post to telephone, teletex, packet switching networks and wide-band cable.

The other experiments in the Commission's package include electronic delivery of German patents and of European Community documents (by a consortium centred in The Netherlands). There are also four experiments in electronic publishing — the two new, wholly electronic journals, and two existing paper journals (in chemistry and mathematics) which will be given electronic versions.

DOCDEL also includes two "development oriented" projects. One will seek standardized methods for handling complex text and graphics and for developing software for electronic document creation and receipt. The other will develop methods for "low run, on demand publishing", where an author creates a text, sends it to a database on a central computer, from which it could be printed on demand by high-speed laser printer for moderate numbers of copies or sent electronically to local printers anywhere in the world for smaller numbers. All these things are, of course, readily conceivable, but DOCDEL will try to discover what is practical and economic, as well as setting standards in a field that could easily become a nightmare of different, incompatible systems.

DOCDEL's tenth experiment will be in creating an "electronic invisible college", initially linking by microcomputer link over the telephone "a group of experts who normally exchange preprints, converse on the telephone, meet at conferences" in three countries.

Robert Walgate

US research townships

Cambridge votes non-nuclear

Cambridge, Massachusetts

ALTHOUGH the Nuclear Free Cambridge Act was defeated by Cambridge residents in the 8 November election, anti-nuclear groups in at least 40 other US cities and towns are pressing for city ordinances or binding referenda that will declare them "nuclear-free zones". The proposed laws would prohibit research and development on nuclear weapons, or their presence, within city limits, with fines or imprisonment for noncompliance. The issues raised in the controversy over the Cambridge statute will be repeated in the coming months in communities across the country.

Some twenty-nine towns have already declared themselves nuclear-free, but these

ordinances have been largely symbolic, since the communities house neither bombs nor defence contractors. Cambridge was the first city where creation of a nuclear-free zone would actually have halted nuclear weapons research; the chief target of the law was the Charles Stark Draper Laboratory, a major defence contractor that designs missile guidance systems.

Despite the strength of the anti-nuclear weapons movement in Cambridge (an earlier non-binding referendum passed with 74 per cent of the vote), voters were apparently persuaded by claims that the potential consequences for freedom of research and local employment outweighed the statute's emotional and symbolic appeal. The 60 to 40 per cent final count was a victory for a group of academics, labour officials and business people known as Citizens Against Research Bans (CARB), who were joined in their opposition by the presidents of both Harvard University and Massachusetts Institute of Technology (MIT) and by the Draper Laboratory — which hired a West Coast public relations expert to direct the campaign. Contributions from the defence industry were a major influence in the campaign; supporters of the act estimate that they were outspent 20 to 1 by their opponents.

According to Hal S. Scott, a law professor at Harvard and a member of CARB, the wording of the statute was so broad and so vague that a court challenge would have nullified it on the grounds that it infringed rights under the First Amendment to freedom of speech. Nonetheless, the law would have had a "chilling effect" on a variety of research projects and on the

willingness of new high-tech companies to set up shop in Cambridge. The most worrying point, said Scott, was that Cambridge residents would have had the right to sue alleged violators for damages or to force a halt to their work. The law could also have set a precedent for bans on other types of research, Scott said.

Even so, Mobilization for Survival, the anti-nuclear group that drew up the statute, believes that constitutional arguments against the act were a "smokescreen" for efforts to preserve lucrative defence contracts. Although admitting that the language of the statute could be "firmed up", it insists that the right to conduct basic research would be preserved. Despite its defeat, the group is delighted with the national press coverage. It claims that its campaign has focused the anti-nuclear movement's efforts to educate the public, and demonstrated that an individual community can "take on the arms industry".

Opponents of nuclear weapons have nevertheless introduced a new element into future nuclear-free statutes, so that towns without defence industries can exert more than a symbolic effect. Divestment clauses now appear in a number of the proposed laws which would require the sale of any investments held by the town in industries that conduct nuclear weapons work.

In the next campaign likely to make headlines, a proposal very similar to the Cambridge statute — but including a divestment clause — has been placed on the ballot in the city of Santa Barbara, California. The city is home to several defence contractors, which are likely to offer stiff opposition before the vote due next year. Supporters say they are confident that if it passes, the law will survive a constitutional challenge.

Christopher Earl

Bio-patent suit

Washington

A LAWSUIT charging two genetic engineering firms, Genentech and Chiron, with infringing a patent for the production of blood-clotting factor VIII was filed two weeks ago by Scripps Clinic and Research Foundation and Revlon, Inc.

Factor VIII is the main plasma fraction used to treat haemophiliacs. It is currently produced by fractionating blood plasma obtained from human donors, a procedure that carries a risk of exposing the haemophiliac patient to hepatitis and, according to recent evidence, acquired

"There's no room for sentiment in the blood business...."



immune deficiency syndrome (AIDS). The patented process, which arose from the work of two Scripps researchers, Theodore Zimmerman and Carol Fulcher, uses monoclonal antibodies to produce pure factor VIII-C.

Ernest Lipscomb, Revlon's director of patents, said last week that the company is examining the possibility of taking similar action against several other companies as well. Revlon, which holds exclusive licence on the patent, currently markets factor VIII derived from human plasma. Neither Genentech nor Chiron has a similar product on the market.

Stephen Budiansky

Medical ethics

Rules for fetal research adopted

Canberra

THE National Health and Medical Research Council (NH&MRC) has taken a bold step in adopting a recommendation by its new Medical Research Ethics Committee to approve experimentation on a "previable fetus" — defined as one that "has not attained a gestational age of 20 weeks and does not exceed 400 grams in weight". In a report on ethics involving the human fetus and human fetal tissue, the ethics committee stipulated that the fetus should be available for research "only as a result of separation by natural processes or by lawful means", and that "dissection of the fetus should not be carried out while a heartbeat is still apparent or there are other obvious signs of life". These stipulations are in addition to the general NH&MRC guidelines for human experimentation.

Transplantation and tissue culture were unexpectedly seen to be in a special cate-

gory, requiring specific consent by the mother and, where practicable, the father, for the somewhat obscure reason that the parents might be concerned to "lose control of their genetic material and its potential". In addition, the relevant institution must keep a complete record of all attempts to transplant human fetal tissue. The transplantation of fetal pancreatic islets of Langerhans cells being developed in Australia as a possible treatment for diabetes would be caught by the new rules.

The report was careful to stress that the responsibilities of clinicians and of research workers should be clearly separated and not vested in the same individual. Thus the clinician should have no interest in the outcome of research nor in the suitability of fetal tissue for it, while the research worker should not be responsible for deciding, for example, whether the fetus is "previable".

Vimala Sarma

British forests

More trees flourish now

THE myth that Britain's tree cover is rapidly disappearing has taken a knock. A census has now shown that land area under woodland in England and Wales has increased substantially since 1947. Furthermore, the total area of broadleaved woodland — a major concern of wildlife conservationists — has remained virtually unchanged over the same period.

The reference date for the census, carried out by the Forestry Commission, is March 1980. The percentage of land area under woodland in England is now 7.3 per cent, while the corresponding figure for Wales is 11.6 per cent. In 1947 the figures

Protection of Rural England, has similar worries about the way mixed stands were classified in the census. But Mr John Kennedy of the Forestry Commission estimates that the classification scheme will if anything tend to underestimate the amount of broadleaved woodland, because conifers in mixed stands that will eventually be entirely broadleaved are removed over a period of time. He also says that many of the conifer plantations managed by the commission, which are

vilified by some conservation groups, are in areas that would otherwise remain as unproductive moorland.

The Forestry Commission's primary aim is to manage timber resources, so it is perhaps hardly surprising that it does not always see eye to eye with conservationists. But there is a general recognition that ancient seminatural and coppiced woodland is being lost in Britain at the expense of habitats supporting fewer species. The Nature Conservancy Council is undertaking a survey of the problem and a Forestry Commission working party will report shortly on a policy for broadleaved woodland.

Tim Beardsley

Soviet agriculture

US sees signs of improvement

Washington

THE Soviet Union's "Food Programme" appears to be making small but real progress towards reducing the country's dependence on imported grains and increasing domestic production of meat, according to the US Department of Agriculture (USDA).

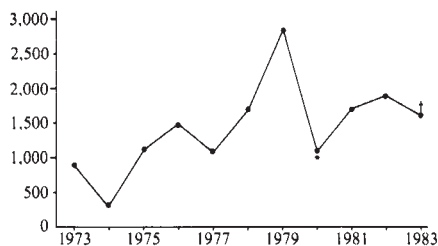
USDA estimates that the Soviet grain crop this year will reach 200 million tonnes, which, together with substantial increases in forage production, is expected to push total agricultural output above the record 1978 level. The greatest gains are reported to be in non-grain animal feeds — pasture, silage and hay — as part of the Soviet effort to boost chronically short supplies of meat. The Soviets also appear to be putting a new effort into production of pulses — peas and beans — which are needed to balance the protein content of animal feeds. Of the 6.6 million tonnes of soybean meal or equivalents now used for this purpose, roughly half is imported.

The Food Programme, which was officially adopted in May 1982, is intended to increase *per capita* consumption of meat, dairy products and fruit. Soviet leader Yuri Andropov this summer reaffirmed the Food Programme's goal of providing these high-quality foods with the "greatest possible self-sufficiency".

The Soviet press has been quick to attribute this year's gains to the success of Food Programme policies, although in fact it may be too early for these policies to have had a real effect. Some Western analysts also question the viability of some of the key components of the programme that appear to run counter to prevailing ideology. These include decentralizing control of agriculture management through the establishment of county-level planning associations (RAPOs is the Russian acronym) and the encouragement of private plots, which currently produce 30 per cent of the country's meat, milk and eggs, and 50 per cent of its fruits on less than two per cent of the farm land. According to USDA, another obstacle appears to be the 1981–85 five-year plan,

which calls for sharp cuts in investment in agriculture-related industries, such as fertilizer manufacturing.

The five-year plan notwithstanding, production of fertilizer in the first nine months of this year was reported to be 9 per cent higher than in the same period last year; production of machinery and pesticides was up 6 per cent. And according to the Soviet Agriculture Minister, a "collective contract" wage system, designed to provide incentives for real increases in farm production, is rapidly being introduced. Under this system, teams of workers are supplied with land, equipment and supplies, and are paid wages as an advance against the final harvest, rather than on a piece-work basis



US agricultural exports to the Soviet Union (\$million). *Partial embargo imposed; + projected.

(for example, according to the number of acres ploughed by a tractor operator). The number of collective contract teams has reportedly doubled since May, and now farm 40 million hectares, some 18 per cent of agricultural land in the country.

USDA is projecting a generally declining demand for grain imports by the Soviet Union as the domestic production of forages increases. Soybean meal will still be in demand to balance feeds, however, with imports expected to grow to 5 million tonnes by 1990. Meat imports are expected to fall by a half.

At the same time, USDA foresees a possible new market in the Soviet Union for imported agricultural technology in the form of machinery, pesticides and herbicides, hybrids and breeding stock if the commitment to increased domestic production continues. **Stephen Budiansky**

IMAGE
UNAVAILABLE
FOR
COPYRIGHT
REASONS

Flourishing elms

were 6.2 per cent and 6.8 per cent respectively. Broadleaves account for 57 per cent of woodland area in England and 30 per cent in Wales.

Conservation and amenity groups were quick to argue last week that figures were not able to convey the change in character of much of Britain's woodland. According to the census, there has been a considerable loss of oak woodland in England and Wales. Dr George Peterken of the Nature Conservancy Council, while welcoming the census results, points out that much of the new broadleaved woodland replacing it consists of pioneering species such as ash, sycamore and birch, as well as scrubby species like hawthorn and willow. Some of these undergrown conifer stands planted by the Forestry Commission but, owing to the way mixed stands are classified, are nevertheless recorded as broadleaved woodland. Peterken also says that differences of definition between the present census and the 1947 study make detailed comparisons difficult.

In 1947 there was a large area of "felled woodland" which was broadleaved in character, while in 1980 felled woodland was largely coniferous: Peterken estimates that, if this is taken into account, an additional 57,000 hectares of broadleaved habitat has been lost beyond that visible in the census figures.

Mr David Conder, of the Council for the

Kenneth Scowen

Making news

SIR — In his article on possible triggering of solar oscillations by gravitational waves (*Nature* 20 October, p.665), Robert Walgate implicitly raises the question of journalistic sensationalism when dealing with scientific questions. Concerning the *Le Monde* headline which he quotes, I asked for a cautious title, or at least the insertion of a question mark as in Walgate's article, obviously without success. Walgate goes on to make an error that I wish to correct. A journalist ought to know that it is impossible for anyone to show a proof of the front page of a Parisian evening newspaper in Frascati several hours before publication. Moreover it would have been absolutely ridiculous for any scientist to do so.

I did show, however, two pieces of information that were not available in the scientific literature when they were published in newspapers and which really did play an important role in our research. One dealt with the identification of the optical counterpart of Geminga and its astrophysical consequences (*Le Monde* 24

lation on gravitational waves and Kotov's claim about earthquakes are reported (*Nature* 304, 689; 1983). Thanks to Gough and *Nature's* speed, someone specializing in Geminga or gravitational waves could have found there a glimpse of the idea that led us to search for periodicity in Geminga, not needing to rely entirely on newspapers for quick scientific release.

This was the only message that I wanted to convey when I held up a cutting from an inside page of an old copy of *Le Monde*.

PHILIPPE DELACHE

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AIDS funding

SIR — In your leading article "Money for AIDS" (*Nature* 25 August, p.671) you ask . . . "Will the money be well spent or are all the haste and dollars a recipe for much poor research, an easy ride on the bandwagon?" You continue . . . "There is a temptation to compare these events with the great drive against cancer initiated by President Richard Nixon in the early 1970s. As some predicted and as many concluded, the huge sums of money made available through the National Cancer Institute (NCI) bought a lot of poor research, and on the whole, poor returns." I do not quarrel with the prediction, but the statement "as some predicted and as many concluded" is in my opinion a sweeping statement for which the evidence is at best anecdotal. I know of no place where the conclusion was drawn that the huge sums "bought a lot of poor research, and on the whole, poor returns". In fact I think it bought a lot of good research and the returns were substantial. Perhaps I am biased. I was a senior official in NCI during this period and played some role, largely intramural, some external in the allocation of the money made available to NCI.

The next thing is that the drive was not initiated by President Nixon; it originated from a panel of consultants on the "Conquest of Cancer", which was a consultative body appointed by a Senate committee. It was also known as the Yarborough Committee. I am not certain where Senator Yarborough derived the idea of creating such a panel, but the recommendations of the panel led to increased appropriations for cancer. I think it would help if you would read the statements that Mr Nixon made at the time he signed the act. He understood as well as the scientists what could be achieved.

I find it difficult to accept that an editorial in *Nature* could continue to repeat charges that really have no substance, but more particularly that the conclusions about whether the research that was done was good and the returns poor are without grounds other than opinion.

NATHANIEL I. BERLIN

Cancer Centre, Northwestern University,
Chicago, Illinois 60611, USA

Indian institute idea

SIR — We share the concern of scientists in India about the plans of the Indian Government to build a "technology city" for Indian scientists trained abroad at a reported cost of \$125 million (*Nature* 29 September, p.350). We suggest that the money be used, instead, for building a new institution for biochemicals and "biotechnicals".

First, this institution would supply India's scientists with these materials. This may be accomplished as follows:

- (1) By regularly assessing the needs of scientists all over the country in order to prepare a list of all biochemicals and biotechnicals needed.
- (2) By meeting these needs either by manufacturing the required materials or by importing them from abroad.
- (3) By providing or making arrangements to provide from a supplier whatever biochemicals, immunochemicals, radiochemicals or biotechnicals are needed within 24 hours of a request from any part of the country. If necessary, a special postal service for scientific supplies could be created.

A second important function of the proposed institution would be to conduct workshops and short courses in specialized areas to give further training to scientists and technicians from other institutions in the country. Faculty for such workshops should be drawn from all over the world.

We further suggest that the proposed institution be built in the city which the government of India may offer as a site for the UN biotechnology centre. Perhaps that will meet the criticism of the UN expert committee that India lacks the scientific infrastructure required by a UN biotechnology centre (*Nature* 29 September, p.350).

BEERELLI SETHI

SUHAS H. PHADNIS

N. SHAMALA

Washington University School
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The Yogi in Nature

SIR — Happy though I am that you should publish Mr E.J. Zuiderwijk's letter (*Nature* 27 October p.758), I trust you will not be influenced by his opinions. Whether the Maharishi Mahesh Yogi is, as he suggests, a clown remains for me a matter of conjecture; I have never met the Maharishi. Like your correspondent I find some of the Maharishi's published views difficult to reconcile with my own. I also recognize an editor's prerogative to publish or withhold. I most strongly reject, however, the philosophy that leads Mr Zuiderwijk to advocate denying access to your columns. Suppression is an ugly word. You are after all, Sir, the Editor of *Nature*, not *Pravda*.

N.H. KEIR

3 Hawkesbury Close, Hartburn,
Cleveland, UK

— MERCREDI 12 OCTOBRE 1983



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Is biology now part of physics?

Reductionism is almost a dirty word, especially in biology, but after thirty years of DNA, it is high time that biologists paid attention to the question of what constitutes an explanation.

In the preface to the first edition of his great book on quantum mechanics, P.A.M. Dirac announced (in 1929) that physics and chemistry had become branches of applied mathematics. And although the sentence was significantly omitted when the second edition appeared in 1931, there is a sense in which Dirac's first claim was correct. For as events have shown, few parts of physical science are untouched by the microscopic view of reality for which the 1920s provided the capstone.

Naturally, classical mechanics still has practical uses, but even with seemingly classical problems, people are increasingly driven to microscopic considerations to check the plausibility of measured parameters. Moreover, it would be absurd to pretend that the most literal reading of Dirac's remark, that all macroscopic phenomena are accurately calculable from first principles and the parameters of the microscopic entities involved, will ever be valid.

The sense in which Dirac's remark has force is that there is reasonable confidence that the surviving impediments to the microscopic explanation of macroscopic phenomena are of two kinds only — computational difficulties and sheer ignorance. While computational difficulties are usually and easily forgiven, their obduracy does of course explain why there is no danger that moderate reductionism, the notion that the properties of wholes are functions of the properties of their parts which are themselves (give or take a quark or so) well known, will put macroscopic physical science out of business. That, in any case, would be assured by persisting ignorance, which appears to be of two kinds — ignorance of previously unknown phenomena (such as the shortage of neutrinos from the Sun) and ignorance born of failures of imagination (such as the compatibility of the physical phenomenon called chaos with the nonlinear deterministic equations that describe them). But with these qualifications, the weak reading of Dirac's claim makes sense and, indeed, has been a powerful stimulus of physical science in the past half-century.

So how soon will it be applicable to biology? At any time in the past thirty years, it might have been held that with the structure of DNA described (and confirmed by experiment), and the mechanisms of protein synthesis known, biology has also been made a branch of applied mathematics. But this is clearly far from

being the case. For all the confidence with which nucleic acid polymers are described in high-school textbooks, even the simple physical chemistry of the double-stranded molecule leaves much unexplained. Empirically, quantities such as the enthalpy of hydrogen bonding between two strands can be worked out, but little is known about the corresponding interaction of single strands of DNA with water while almost next to nothing has been done to take account of the interactions between consecutive pairs of bases in a double-stranded molecule (which must nevertheless account for the differences of structure between left-handed helices and the normal right-handed variety). More generally, and more worrying, the interaction of large molecules with the watery media in which they are usually found is not so much a puzzle as an issue tacitly recognized to be too complicated to tackle and best for the time being ignored.

In much the same way, the search for an explanation of how enzymes function is usually conducted on principles that fall short of full-blooded microscopic explanation. To be sure, there is now a long list of enzyme molecules whose interaction with the ligand molecule has been reasonably well inferred and whose catalytic effects can be explained by some supposed change of shape, an allosteric transformation. This, however, is a far cry from what might be thought proper if biology were already a part of physics — in principle, the prediction from the specification of some protein molecule of its function in real life. Moreover, it is plain that the difficulty cannot simply be written off as computational: the gap between what (elsewhere) can be calculated and the behaviour of apparently bistable molecules is too great to be spanned without a substantial knowledge empirically arrived at about the functioning of molecules that act as enzymes.

Exactly similar difficulties afflict other now familiar studies, the processing of DNA in cells for example. In bacteria, the characteristic places on DNA molecules at which transcription starts and, then, stops (and which thus define a gene) are known, but only in the sense that they have been recognized for what they are. While ignorance persists about the way in which the associated enzymes function, no would-be reductionist can write off the problem as a computational difficulty. And, as is well known, eukaryotic organisms present more serious problems. Who would pre-

tend that given a sufficiently large computer, he could for example tell which parts of a stretch of DNA are functional and which are, outwardly at least, irrelevant?

The energetics of identifiable biochemical processes in cells points to a different kind of problem. The explanation of how cells can maintain large concentrations of materials such as ATP well away from what would be the natural condition of equilibrium, or transfer protons across cell membranes (as in oxidative phosphorylation) or sodium and potassium ions in different directions across neuronal membranes, can be described in terms consistent with thermodynamics. That is what non-equilibrium thermodynamics is for. But while the practitioners are confident enough when calculating (or saying that they could calculate with sufficient computing power) the voltage of a thermocouple (a simple system out of equilibrium), even the simplest processes within cells can be accounted for only by taking for granted the existence (again far from chemical equilibrium) of the complicated entities in which they occur.

This is why the reductionists are often opposed by those who argue that while their programme may work in physics, it is bound to be defeated in biology not merely by the lack of credibly sufficient computing power but by the sheer complexity of living systems. Complexity makes a qualitative difference — and, in any case, the search for microscopic explanations of macroscopic happenings is, in this case, a poor guide to the design of fresh experiments and observations.

But is that necessarily the case? In physical science, the search for microscopic explanations has been remarkably productive in the past half-century, especially because people have sensibly not followed the bigoted course of pretending that only microscopic explanations matter. The truth is that microscopic explanations are in principle simpler, and often more stimulating as well, than those which are essentially correlations between one set of phenomena (effects) and another ("causes"). Molecular biologists might prudently keep those benefits more clearly in mind than seems customary in the present rush to compile what might be called the natural history of the gene, if only because the only possible answer to the question at the top of this page is "not yet".

John Maddox

Particle physics

On the scent of glue

from Frank Close

DO glueballs exist? A series of theoretical and experimental developments this summer has sharpened the controversy and produced some new paradoxes. But a new paper from C. Edwards and colleagues (*Phys. Rev. Lett.* **51**, 859; 1983) and reports at recent international conferences now add some quite new, if circumstantial, evidence for their existence. Something definitely seems to be abroad — but is it glue?

Known nuclear material is built from quarks, the interquark forces being transmitted by gluons — analogues of the photons associated with electromagnetic forces. Photons are electrically neutral so they do not cluster together to form exotic new matter. Gluons, by contrast, are mutually attracted by the same forces that act on quarks, so they can not only transmit the forces but also feel them directly. Thus gluons should not voyage freely through space but should mutually attract and aggregate until the forces are neutralized. Exotic 'glueballs' should exist, as should new combinations of quarks and gluons. In these latter hybrid states the forces on the quarks alone are not neutralized; gluons rather than more quarks are attracted to the system until neutralization occurs.

The theoretical interest in glueballs has been around for a decade, but evidence for their existence is sparse and its interpretation still in dispute. The original predictions for the masses of the lightest glueballs were made in 'bag' models. These were inspired by the empirical observation that quarks and, presumably, gluons are permanently confined within a region of space of radius 1 fermi (10^{-15}m). Allowing quarks to be almost free within this domain, but forbidden to cross the surface, imposed boundary conditions on the quark wavefunctions and in turn forced them to exist in specified eigenstates. Three massless quarks so confined turn out to have an energy of about 1 GeV — the proton's mass. The theory of interquark forces, quantum chromodynamics, can be applied perturbatively within the bag and the shifts in energy of the clusters computed. A reasonably good picture of subnuclear particles has been developed since 1975 and the study of quantum chromodynamics and quarks in bags is of increasing interest to low-energy and nuclear physicists. What works so well for quarks should do so for gluons too with the Dirac equation for spin $\frac{1}{2}$ quarks being replaced by Maxwell's for spin 1 gluons, given the approximation that they are almost free within the bag. The masses of the lightest glueballs turn out to be around 1 GeV.

The fact that the gluons mutually interact is then taken into account by

calculating the quantum chromodynamic forces acting on them within the bag. The resulting shifts in energy are analogous to the hyperfine shifts in atomic systems and depend upon the total spin of the glueball. Although theorists argue among themselves as to the precise magnitude of the shifts there is general agreement that two spinless glueballs lie lowest in mass, the next heaviest having spin two (in units of Planck's quantum $\hbar$). The lightest spinless state should have positive parity (the sign of its wavefunction being preserved under mirror reflection); the heavier spinless glueball having negative parity (mirror wavefunction has opposite sign).

There is a growing effort to study quantum chromodynamics in a world where space-time is a lattice of discrete points rather than a continuum. This, in principle at least, allows computer-aided but model-independent computation of glueball masses. There are doubts as to whether the lattices used are large enough to do such a job meaningfully but, that aside, the results are most exciting. Indeed, the pattern found in the bag model studies was also reported in lattice calculations by M. Teper (University of Annecy) at the European Physical Society conference in Brighton during July.

Similarly, about a year ago there was a surge of interest in the properties of hybrid states consisting of quarks and gluons. In bag models the lightest of these states is expected around 1.5 GeV and the first calculations of such states have now been made on lattices. The lattice spectrum is rather richer than that found in bag models but, for the lightest states, the bag and lattice expectations are very similar.

Everyone agrees that the lightest glueballs and hybrids all have wave functions that preserve sign under the action of charge conjugation. This has the consequences that these states can be produced in conjunction with a single photon in decays of the massive psi particle — a bound state of charmed quark and a charmed antiquark.

As the psi particle can now be produced to order in electron-positron collisions, over a million events have been accumulated at Stanford, California, and at Hamburg's electron-positron storage ring. Studies of $\psi \rightarrow \gamma + \text{'something'}$ have become quite detailed; examination of the properties of the 'something' (X) will, it is hoped, provide evidence for gluonic matter. The iota state, mass 1.4 GeV, found this way in 1979, was the first *prima facie* glueball candidate. And, indeed, it is in such experiments that some unexpected new phenomena have been announced this summer that at least give a hint of glue.

C. Edwards and colleagues (*Phys. Rev. Lett.* **51**, 859; 1983) report that if X = two pions and an eta particle, clear evidence is seen for a hitherto unknown resonance at 1.75 GeV. This accompanies the earlier discoveries of iota (1.4 GeV) and theta (1.65 GeV) in psi decays. Unpublished and preliminary data reported by D. Hitlin (Stanford Linear Accelerator Center) during the high-energy physics conference at Cornell in August show that when X = a photon and two pions, structure is seen around 1.4 GeV which is more than a simple iota. Complicated structures were also seen when X = two pions and an eta, additional to those of Edwards *et al.* At least one and maybe two further states are lurking hereabouts if the totality of data is to be believed. If this abundance survives further investigations then it may be hard to accommodate them all without going beyond the conventional quark model — gluonic or hybrid states may need to be invoked.

Most exciting of all the new studies was the report by K. Einsweiler (Stanford Linear Accelerator Centre) at the Brighton European Physical Society Conference. When X = two kaons a prominent new resonance of mass 2.2 GeV and unexpected metastability was seen in the SLAC Mark III detector. Its width is comparable to or less than the resolution of the experiment; its lifetime is at least five times greater than for conventional quark matter at such a mass. One hypothesis put forward to interpret this anomaly is that it is the long-sought Higgs particle. This seems unlikely in view of its copious production rate; Higgs particles should be hardly visible in $\psi \rightarrow \gamma X$, not singularly prominent as Einsweiler's state appears to be.

If this is indeed a glueball then it ought to show up in channels other than simply when X = two kaons. There is no evidence for it in the two-pion channel; other modes that it could appear in have not yet been studied in enough detail at this mass for us to be able to say one way or the other. The community will be watching development of data on this state with interest.

For decades experiments were performed with pions and protons: hurl quark clusters at one another and quark clusters emerge. By these techniques the spectroscopy of quark states was disentangled and the quark model of hadronic matter verified. Now we can study psi decays in which initial quarks and antiquarks are totally destroyed so that no memory of them remains but for their energy. This energy must consist of the quanta of the force fields associated with the quarks charges — electrical and colour — namely photons and gluons. This is something new and if one continues to find prominent signals never before seen, the onus is increasingly upon people to show why they are not evidence for gluonic excitations.

One possibility is that the state seen in the SLAC Mark III detector is almost pure glue, setting the mass scale for glueballs at 2 GeV, something that has been argued for

by the Moscow school and which may be consistent with lattice calculations, given their inherent uncertainties in the string tension (a measure of the overall energy scale). Hybrids are only half glue and their masses would then lie in the 1–2 GeV regions, midway between glueballs and the lightest quark states in line with their hybrid constituency. This could help explain the proliferating structures in this region.

With psi decays we have an unparalleled opportunity to study gluonic excitations. In Europe the LEAR facility at CERN will study low-energy annihilations of protons and antiprotons and, again, should generate gluonic excitations along with quarks. The fundamental importance of

establishing gluonic structures cannot be stressed too much. As Kurt Gottfried (Cornell University) at the European physical Society conference pointed out, simply staring at the hamiltonian of quantum chromodynamics would not help one deduce the properties of glueballs any more than staring at the analogous hamiltonian of interacting electrons, nuclei and photons would tell one that solids exist. Yet everyone knows that solids exist; it would be a shame if the existence of glueballs could only be appreciated by a privileged few who have devoted a lifetime to studying hadron spectroscopy. □

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Molecular biology

Enhancers, chromosome position effects, and transgenic mice

from Brigid Hogan

It is now two years since the first reports that cloned DNA microinjected into the pronuclei of fertilized mouse eggs can be stably integrated into the chromosomes of the developing embryo and passed on through the germ line of the adult mice. The generation of 'transgenic' mice was seen as a way of analysing both the developmental regulation of individual genes, and the biological consequences of the expression of natural and specifically engineered gene products. Following these early reports, there have been mixed fortunes for the relatively few laboratories set up to generate transgenic mice. On the one hand there have been notable successes, in particular the 'supermice' produced in Ralph Brinster's laboratory in Philadelphia. These grow to a large size as a result of synthesizing high levels of growth hormone from integrated DNA consisting of the rat growth hormone structural gene coupled to the mouse metallothionein-1 promoter. On the other hand, other laboratories have obtained only very low frequencies of transgenic mice from injected eggs, or have had good yields but little or no expression of the integrated genes^{2,3}.

Resolution of the problem of the tissue specific expression of integrated genes has been brought closer by two recent papers. In the first³, Elizabeth Lacy and her colleagues present a detailed analysis of five separate lines of mice transgenic for DNA containing the rabbit β globin gene, β_2 pseudogene and flanking sequences. Their results suggest that for these (and other) globin genes, chromosomal location is dominant over any *cis*-acting control sequences brought in with the DNA. In contrast, in this week's *Nature*⁴ (see p.332), Brinster *et al.* suggest that in the case of the rearranged α immunoglobulin light chain

gene, a tissue-specific enhancer sequence within the gene can override chromosomal position effects, and direct high levels of gene expression in the spleen but not the liver of their transgenic mice.

Before discussing these results in detail it is worth noting that it is still not known what determines the frequency with which transgenic mice are produced. Even in laboratories that have gained considerable experience this can vary between three per cent and more than 40 per cent of live offspring³, and may be a function of the amount of DNA injected, or the form in which the cloned DNA is excised and presented. What is clear is that even when linearised fragments of plasmid DNA are injected, most of the integration sites contain multiple (usually 10–40) copies, in head to tail tandem arrays. When and how integration occurs, and what determines the selection of one or two chromosomal sites per embryo, out of those potentially available³, remain open questions.

The immunoglobulin light chain gene injected by Brinster *et al.* consists of a kappa α variable segment (V_α) already rearranged next to a joining region (J_α) which is separated by several kilobases of sequence from the downstream unique constant region (C_α). Rearrangement of one of the ~ 10 possible V_α regions next to one of the J_α regions normally takes place in pre-B cells during the differentiation of the B lymphocyte lineage. A similar rearrangement of a heavy chain variable segment (V_H) next to a heavy chain joining (J_H) region also occurs in these cells, but at an earlier stage. Recent work with both rearranged light^{5,6} and heavy^{7–9} chain genes has shown that they are not efficiently or correctly transcribed from the promoter in front of the V gene when transfected into non-lymphoid cell lines: efficient trans-

cription is only obtained when they are transfected into lymphocyte-derived cells. Deletion analysis has located the DNA sequences responsible for this tissue-specific, high-efficiency transcription to a short region within the intron between the J and C genes. These sequences fall into the category of 'enhancers' — short stretches of DNA containing a core of about 8 nucleotides (see refs 7, 10 for references) that can act over a distance of several kilobases to increase transcription from promoter sequences.

Usually enhancers are located to the 5' side of promoters, but experiments in which they have been deliberately relocated or reversed show that their specific orientation is not important; and clearly in the case of the immunoglobulin genes they can normally act on upstream promoters. How cellular enhancers work is not yet known but one idea is that their structure makes them efficient entrance sites for RNA polymerase, and that their activity can be controlled by tissue-specific binding proteins. At present the best studied enhancers are those associated with viruses and the immunoglobulin genes. New evidence, from Michael Walker and colleagues at the University of California, San Francisco, which will soon be published in *Nature*¹¹ shows, from short-term assays, that there are also tissue-specific enhancers near the 5' end of insulin and chymotrypsin genes.

The work of Brinster *et al.* strongly suggests that the α light chain enhancer is a particularly efficient one since it appears to work in a tissue specific manner even when the gene is stably integrated into several different locations. Just how well this conclusion stands up will have to await the analysis of more transgenic mice, and a wider range of tissues than just spleen (presumably the B lymphocytes within it) and liver. In the case of the rabbit β globin gene used by Lacy *et al.* it seems that any control elements brought in with the DNA cannot override chromosome position effects. In none of their five lines of transgenic mice is the rabbit globin gene expressed in erythroid cells. However, in one line it is transcribed at a low level in muscle and in another in testis. In both cases transcription is from the rabbit globin promoter and is not the result of aberrant readthrough.

One explanation of their results is that the injected DNA did not include any erythroid cell-specific enhancer, so that transcription only occurs efficiently under the influence of any host cell enhancer the gene happens to integrate near to. Alternatively, tissue-specific controls may be present on the injected DNA but are not efficient enough to counteract local chromosome position effects. The idea that tissue-specific control elements may have different levels of efficiency could account for variations in the expression of the chicken transferrin gene in transgenic mice recently reported by McKnight *et al.*¹². In most mice the chicken gene is

expressed predominantly in the liver, where the endogenous gene is also most active. In some mice, however, tissue specificity is not so clear cut.

As more is learnt about how enhancers work in relation to chromatin structure and nuclear environment it may be possible to make correlations between the integration site and the level of activity of foreign genes. This in turn may enable the genetic engineer of the future to select the right combination of enhancers, promoters, and flanking sequences that will ensure efficient tissue specific expression of a gene

wherever it happens to integrate. □

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Evolutionary biology

Brain size, energetics, ecology and life history patterns

from Paul H. Harvey and Peter M. Bennett

IN HIS *Descent of Man* Charles Darwin wrote that "False facts are highly injurious to the progress of science, for they often long endure; but false views, if supported by some evidence, do little harm, as every one takes a salutary pleasure in proving their falseness"¹. Until recently the interpretation of variation in relative brain size among mammals was dominated by a false fact — an incorrect value of the exponent scaling brain weight to body weight. Now, alternative hypotheses attempting to account for this variation are being tested from an ever-widening data base.

Along with Stephen Gould writing in 1975, most scientists have thought that brain weight "generally scales to the two-thirds power of body weight (an immense number of sources ranging from Snell, 1891 [ref. 2] to Jerison, 1973 [ref. 3]); a relationship between brain weight and body surfaces is here implicated, but it has never been satisfactorily explained"⁴. More recent analyses, using a larger number of mammalian species, show that the exponent is not two-thirds but approximately three-quarters⁵⁻⁹. This suggests a quite different category of solutions. While a two-thirds exponent implicates body surface areas (as they increase with the two-thirds power of body weight), so a three-quarters exponent suggests an energetic explanation (basal metabolic rates, measured as energy needs per unit body weight in a given time period, increase with the three-quarters power of body weight).

Two hypotheses have been proposed to explain the new relationship. The first, by Este Armstrong, published recently in *Science*¹⁰, points out that brain size scales against metabolic needs (the product of metabolic rate and body size) with an exponent of one and argues that "among extant mammals an increase in brain size keeps pace with an increase in body size when the size is adjusted for the availability of energy". Presumably, for whatever

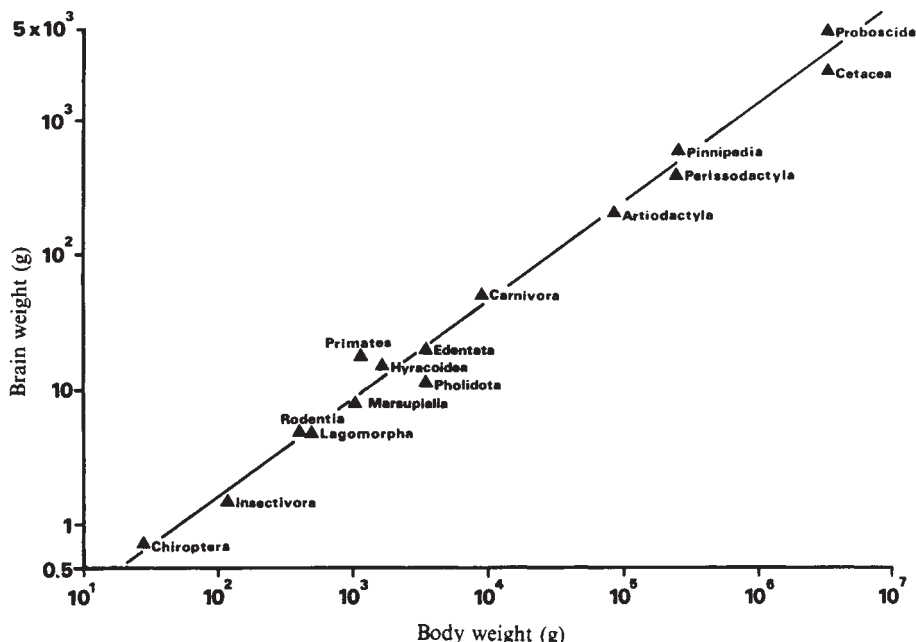
reason, mammals have been selected to supply a constant proportion of their energy needs to the brain. There is no suggestion as to why this should be so. But some mammals, notably the primates, lie above the typical mammalian line and thus have larger brains for their metabolic needs than most other mammals. The argument here is that primates allocate a higher proportion of their body metabolism to their brains (9–20 per cent) than do other mammals (about 5 per cent). But this is not an explanation, merely a restatement of facts — if primates have relatively large brains, then they will need more energy to keep them working.

The second hypothesis published two years ago in *Nature* by R.D. Martin⁶ is cited but not discussed by Armstrong. Martin noticed that although the brain/body exponent was about 0.75 for

mammals, it was about 0.56 for cross-species plots of both birds and reptiles. He claimed that, since the metabolic exponent for all three groups is near 0.75, "there is no case for suggesting a direct link between the brain size of the adult and its own metabolic rate". Instead, he argued that on an evolutionary time scale the amount of energy the mother can put into the neonate might be the limiting factor on adult brain size. Since birds and most reptiles lay eggs, the "metabolism of the egg then provides for development of the embryonic brain"¹⁶. As a consequence, the exponent linking maternal size to neonatal brain size would be the product of that between mother's metabolism and egg size (0.75) and that between egg metabolism and neonatal brain size (0.75), which is about 0.56. Post-natal growth in brain tissue is generally a result of the addition of glial cells and the expansion of existing neurones. We might, then, expect isometry between neonatal brain size and adult brain size. If this is so, the exponent relating adult brain weight to body size would be 0.56 in birds and reptiles, and 0.75 in mammals. Therefore, a prediction of Martin's hypothesis is that neonatal brain size should be a constant proportion of adult brain size, whatever the species. Within the mammals this prediction does not quite hold, but one cause for this discrepancy may lend strength to Martin's hypothesis.

Mammals can be divided into altricial and precocial forms. The latter are characterized by species with particularly long gestation periods and, therefore, large neonatal brain sizes for the maternal body weight. Adult precocial mammals have larger brains for their body sizes than do adult altricial mammals. Within each group, as predicted, there is an isometric relationship between neonatal and adult brain sizes⁶. Incidentally, there is one

Allometric relationship between brain and body weight across 15 orders of mammals. Order points are derived from family averages, which are themselves calculated from generic values based on 883 species measurements⁹.



notable deviation from this relationship among precocial mammals (which include primates) — humans^{11,12}, which are characterized by a larger adult brain size relative to their neonatal brain size than other primates.

While developmental constraints can be used to explain some deviations from the overall mammalian brain:body weight curve, other patterns have been related to species' feeding ecologies. Frugivorous (fruit-eating) bats have relatively larger brains than insectivorous species¹³⁻¹⁵; while among the primates and small mammals, folivores (leaf-eating species) have relatively smaller brains than members of other dietetic categories^{16,17}. The common interpretation of these differences is that they "arise through variation in either the range of stimuli that need to be processed for feeding or information storage and retrieval systems, rather than through the nature of the food source"¹⁸. For example, frugivorous primates have larger home ranges for their body size than folivores¹⁹ and may thus need to process, store and retrieve more information. Recent information suggests, however, that an energetic hypothesis may encompass these apparent ecological correlates of relative brain size²⁰. Insectivorous bats and some folivorous mammals tend to have low metabolic rates for their body size²¹⁻²³, and Armstrong¹⁰ shows that when brain size is plotted on metabolic needs instead of on body size, insectivorous and frugivorous bats lie on a similar curve. Unfortunately, she misinterprets the dietetic association with relative brain size in primates to be between insectivorous versus other species whereas, in fact, the major difference is between folivorous and frugivorous species. Folivores have relatively smaller brains than frugivores. The only study reported to date on the relationship between basal metabolic rate and these two dietetic categories in primates compares two species of Old World monkeys²⁴. As expected, the folivorous *Colobus guereza* has a lower basal metabolic rate for its body size than the frugivorous *Cercopithecus mitis*. As more data become available, it will be interesting to see whether species belonging to these two dietetic categories lie on the same curve when brain size is plotted against metabolic needs — both Armstrong's and Martin's hypotheses predict that they will.

One other pattern of deviations from the mammalian brain:body line cannot, apparently, be interpreted in energetic terms. Although brain size increases with the 0.75 power of body size across mammalian species as a whole, when smaller taxonomic units are examined the exponent drops. For example, the exponent among species within genera is usually about 0.5 (ref. 9). This means that smaller bodied species within genera tend to have relatively larger brains than larger species, when relative brain size is measured as deviations from the general

mammalian curve. The reason for this change in exponent value with taxonomic level is not clear, though it could result in part from body size responding more readily to selection in evolutionary time than does brain size^{25,9}. An evolutionary lag may exist until brain size adjusts to the optimal scaling value. One consequence of this phenomenon is that analyses of brain against body size must use a single and appropriate taxonomic level for analysis. The 0.75 exponent, although applying to species within the class Mammalia does not necessarily apply to a comparison among orders within the class. In the figure, we present an inter-order analysis using data on 883 mammal species extracted from a variety of sources^{15-17, 26-35}. The exponent

(measured by major axis analysis³⁶) is 0.72. The recently acknowledged interspecies value, 0.75, lies within the 95 per cent confidence limits but the older rejected value of 0.67 does not⁹.

This corrected value has encouraged the production of alternative hypotheses, some of which make sense of previous observations as well as bringing into the arena new and relevant considerations. It seems that ecology, energetics, evolutionary processes and life history patterns may all be important factors to be considered. □

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Earth sciences

Mixed-up magmas

from R.S.J. Sparks

ONE of the major objectives of igneous petrology is to account for the extraordinary diversity of magmas that erupt on the Earth's surface. Well over fifty years ago petrologists recognised that, in complex multicomponent silicate systems, crystallization from a melt, or partial melting of a rock, must be the dominant processes generating chemical diversity in magmas. Subsequent research on igneous rocks confirmed this view so successfully that other processes — including magma mixing — were often ignored.

Now, times have changed. Magma mixing is receiving a great deal of attention for it is apparent that the majority of volcanic rocks show evidence of mixing between different magmas. Two papers in this issue of *Nature* (Fitton *et al.*¹, p.327; Bell², p.323) provide examples of the kind of detailed geochemical and petrological investigation that have revived the fortunes of magma mixing as a petrogenetic process.

Magma mixing has traditionally been unpopular as an explanation for composi-

tional diversity. First, mixing by its very nature tends to reduce, rather than increase diversity. Second, mixing still leaves the problem of accounting for the end members. Third, in innumerable cases igneous rocks that are closely related in space and time cannot be related to one another by simple mixing of two end members. Nevertheless, a wealth of evidence has emerged in the last few years to show that mixing is probably a fundamental process in magma genesis. Detailed analytical studies of the mineralogy and chemistry of igneous rocks of the kind carried out by Fitton *et al.* and by Bell represent one kind of approach. Both papers show that magma mixing has occurred from mineralogical evidence, such as the presence of xenocrysts, and computer-modelling of crystallization and mixing processes. There are probably now many tens of similar studies, all published in the last few years, which document evidence for magma mixing in igneous rocks.

A second kind of study involves the

thorough documentation of volcanic eruptions, and their products. These studies are the more persuasive since much other geophysical and observational data can be used to enable convincing interpretations to be made. Fitton *et al.*'s documentation of the 1982 Mount Cameron eruption is one example of such a study. Perhaps the best recent documentation of the relationship between volcanism and magma mixing is provided by Soviet scientists who studied the 1975–1976 fissure eruption of Tolbachik Volcano in Kamchakta³.

A surprisingly simple picture has emerged from these studies which, in hindsight, appears all too obvious. Most volcanoes are underlain by complex plumbing systems which allow magma from the mantle to ascend to the surface. Magma often gets caught in traps (magma chambers) on the way to the surface where cooling and crystallization cause differentiation. At intervals, varying from a few years to tens of thousands of years, the system is recharged by new magma rising from depth. As recharge occurs the fluid pressure in the plumbing system and magma chambers increases until the volcanic edifice fails and an eruption begins. In many cases recharge also causes mixing between magmas of different composition in the system by both natural and forced convection. It is thus hardly surprising that so many studies show that magma mixing occurs shortly before or during eruptions. Most eruptions occur because the plumbing system is activated by ascent of new magma.

The Tolbachik and Mount Cameron eruptions provide excellent examples of the general model outlined above. During the Tolbachik eruption, seismic data show how a high temperature magnesian basalt ascended from a reservoir at the base of the crust, 30 km below. A fissure eruption of this basalt began but after a few weeks the new magma entered a high-level reservoir containing already differentiated basalt. A mixture of the two magmas erupted for a while and gradually became richer in the differentiated basalt end member. The high-level chamber eventually drained and caused a 1.7 km diameter caldera to form at the summit of Tolbachik volcano. A broadly similar series of events was seen in the Mount Cameron eruption documented by Fitton *et al.* except that the mixed basanite lava became richer in the more primitive magma with time.

It is likely that most magmatic systems are open. The main cause of volcanic eruptions is the increase in fluid pressure caused by recharge of the plumbing system. Compositional diversity of the magmas is still likely to be mainly brought about by partial melting and fractional crystallization during the long periods of quiescence in the magma reservoirs beneath the volcano. Magma mixing will, however, be an inevitable consequence of the recharge and eruption process, and make interpreting the origin of igneous rocks that much more difficult. The consequences of magma mixing

can be significant and sometimes surprising. Repeated episodes of mixing can generate incompatible trace element compositions which depart significantly from those expected by crystallization or melting⁴. The topology of phase diagrams, where liquid paths are characteristically non-linear, require that mixing can produce compositions which are impossible to produce by fractional crystallization of any particular parental melt. Furthermore such mixed magmas must generally

be superheated or undercooled, which can cause distinctive textures, as well as unexpected orders of mineral crystallization. Mixing is a process that is no longer safe to ignore. □

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Science in Africa

Historical science in Sokoto, Nigeria

from Beverly Halstead

THE northwestern corner of Nigeria, Sokoto State, holds a special place in the history of West Africa, as it was from here that the great Jihad or Holy War took place during the early part of the last century under the leadership of Usman Dan Fodio. It was in this same region that early in the present century fossil reptiles and fish from the uppermost Cretaceous and basal Tertiary were discovered by officers of the Geological Survey of Nigeria and subsequently described in the 1930s by staff of the British Museum (Natural History); W.E. Swinton¹ and E.I. White².

It was not until half a century later that expeditions were mounted specifically to recover further material, those in the 1970s producing the skulls of the mosasaur *Goronyosaurus*, the dyrosaur crocodile *Sokotosuchus* and the pelomedusid turtle *Sokotochelys*. The 1977–1978 International Palaeontological Expedition to Sokoto State ran into severe difficulties as the party was apprehended by the Nigerian security authorities. Happily the misunderstandings were finally resolved amicably, as a consequence of the personal intervention of His Excellency the Military Governor, the late Brigadier Umaru Alhaji Mohammed, who provided all the necessary logistic support. It was the intention to continue such joint collaboration directly with the Sokoto State Government and there was a plan to construct a museum to house and display the collections³.

Since that time, new civilian governments have been elected throughout Nigeria and are now due for re-election. It was not until 1982 that the Sokoto State Government convened an international seminar on the archaeology and early history of Sokoto State under the auspices of the Sokoto State History Bureau — 'history' in this context being taken to include geological and palaeontological history. Sokoto state is unique among the nineteen States of the Federation of Nigeria in having set up a state academic body concerned with the region's history. The first seminar was hosted by them at the

beginning of June 1983 and stressed the importance of bone-bearing horizons in the uppermost Cretaceous and the basal Palaeocene. These have enormous potential in helping resolve the nature of the Cretaceous — Tertiary transition in tropical latitudes. The paucity of Palaeocene vertebrates throughout the continent of Africa also makes Sokoto a region of especial interest. The abundance and importance of fossils was recognized and as a consequence of the seminar it was decided that there should be a place for such palaeontological studies as an integral part of the bureau's future researches and that part of the planned museum should be devoted to the palaeontological and geological history of the State.

The establishment of the Sokoto State History Bureau and its current policies mark a major step forwards in intellectual development in Nigeria and in a sense points the way for the rest of West Africa: a worthwhile way forward for local scholarship, wherever leading politicians have both an awareness of their country's heritage and an appreciation that scholarship is international, and that knowledge should have no barriers of nationality or race.

It is hoped that the academic endeavours of Sokoto State will receive an appropriate welcome and practical support and encouragement by the academic community both in Nigeria and elsewhere. There have been local difficulties in exploiting the fossil riches of Sokoto State but it is hoped that with the enthusiastic nucleus now in Sokoto, these important deposits can be fully studied and the results made available to the international community. In the years to come Sokoto could become an important centre in West Africa for the study of palaeontology. □

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Evolutionary biology

Mitochondrial DNA: new clues about evolution

from Nick Barton and J. S. Jones

EVOLUTIONARY BIOLOGY is largely about reconstructing history. The imperfections of evolution's historical document, the fossil record, mean that past events must usually be deduced from their modern consequences. It was not until it became possible to make antigenic and electrophoretic comparisons of protein structure that there was a chance to sample the genomes of related species and to infer evolutionary history from present patterns of genetic affinity. One of the surprises of the 'find them and grind them' era of evolutionary genetics was to discover that morphological evolution has often proceeded without much change at loci which code for proteins; humans and chimpanzees appear no more distantly related on this measure than are subspecies of other mammals or morphologically indistinguishable species of *Drosophila*¹⁻³.

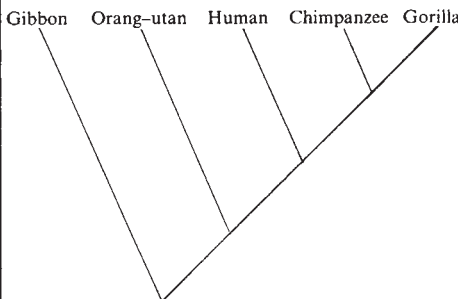
Biologists working on the comparative anatomy of the genome now have a new toy: genetic polymorphism in the DNA itself, which can be detected either by using restriction enzymes to cut DNA at specific points and to produce fragments whose lengths are an indication of individual differences in structure, or by direct sequencing of the DNA molecule. So far, most work has been done on mitochondrial DNA, which has a number of advantages as an indicator of evolutionary change.

The mitochondrial genome of animals is small — about 16,500 base pairs in mammals — and is easy to study⁴. It has been sequenced in man, mouse and cow, and large parts of the sequence are known for *Xenopus* and *Drosophila*⁵⁻⁹. In mammals, mitochondrial DNA shows striking economy of organization, with none of the extensive non-coding sequences found in the nucleus. As the sperm contributes almost no cytoplasm to the fertilised egg, mitochondrial DNA is maternally inherited; crosses between two species of tobacco budworm (*Heliothis*) show that after nearly 100 generations of backcrossing hybrid females to males of one of the parental species, all the mitochondrial DNA is that of the original maternal type¹⁰.

The pattern of mitochondrial inheritance (which involves segregation in mitotic as well as meiotic cell divisions) can itself lead to evolutionary change¹¹. For example, there have been shifts in the mitochondrial genome in a lineage of Holstein cows which occurred within only two generations, and which probably arose from non-random segregation of mitochondria during egg formation¹². In addition, because it lacks efficient repair mechanisms, mitochondrial DNA has a

higher mutation rate than does that of the nucleus¹³. Variation may also result from gene conversion in mismatched genomes in cells containing more than one type of mitochondrion (see this issue of *Nature*, p.400).

All this means that mitochondrial DNA can evolve rapidly. Although the order of mitochondrial genes has been conserved for some 350 million years (since the separation of the lineages which gave rise to frogs, cows, mice and men), there have been alterations in their nucleotide sequence at a rate ten times as great as that in the nucleus⁴. Some parts of the mitochondrial genome have evolved particularly quickly. For three of the 22 mitochondrial tRNA genes the rate of change has been more than a hundred times faster than that for corresponding nuclear genes¹⁴. There is



A new genealogy of the primates produced by analysis of mitochondrial DNA.

also extensive polymorphism within species. Restriction enzyme studies suggest that two randomly chosen humans differ by about 0.4 per cent in their mitochondrial DNA sequence¹⁵, and the amount of mitochondrial DNA polymorphism in other primates may be even greater than this¹⁶. In a 900 base-pair sequence of mitochondrial DNA from seven different humans the average genetic difference between individuals is about 1.7 per cent¹⁷. Comparison of individual patterns of restriction fragments with those of mitochondrial DNA from the single human whose complete sequence is known has identified 163 variant sites throughout the mitochondrial genome and shows that human mitochondrial polymorphisms involve additions and deletions of small DNA segments as well as point mutations^{18,19}.

What makes mitochondrial DNA so valuable in tracing patterns of evolutionary relatedness? First, each mitochondrial clone preserves a distinct combination of linked mutations which evolves independently of others, and can be used to deduce phylogenies. In contrast, muta-

tions at unlinked loci (such as most electrophoretic alleles and nuclear DNA variants) give a more ambiguous measure of overall relatedness. Although polymorphic sequences of nuclear DNA short enough to be safe from recombination or gene conversion over the relevant timespan can retain substantial linkage disequilibrium²⁰ much less is known about the population genetics of closely linked polymorphic loci in the nuclear than in the mitochondrial genome. Maternal inheritance leads to other differences between nuclear and mitochondrial genes. The dispersal rate of maternally inherited genes is that of females, rather than the average of the two sexes, and their population size is lower; patterns of distribution of mitochondrial genes are therefore more subdivided than are those of nuclear genes²¹.

For example, in the North American deer mouse *Peromyscus maniculatus* gene frequencies for enzyme polymorphisms are rather homogeneous throughout its range. Mitochondrial genotypes are, however, strongly patterned into five different assemblages which probably mirror the historical relationships between populations of this species²².

A survey of mitochondrial DNA polymorphism in 112 humans from various parts of the world shows that *Homo sapiens* is much more geographically uniform than is *P. maniculatus*^{14,18,19}. Three of the 35 mitochondrial variants found are present in all human populations while each of the others is restricted to a particular ethnic group. One of the three widespread variants is probably the common ancestor of the other human mitochondrial genomes. This pattern of mitochondrial substitutions has been used to construct a phylogeny of mankind, which shows, for example, that African populations are the most divergent from others and that the amount of mitochondrial differentiation within Africa is notably greater than elsewhere in the world²³.

Changes in the mitochondrial genome can also be used to trace patterns of common ancestry among species and have recently been used to unravel the evolutionary history of man and his relatives. There are considerable differences in the mitochondrial maps of gorilla, orang-utan, gibbon, chimpanzees and man^{16,24}. The gorilla has a deletion of 95 base-pairs, while the gibbon is the most different from man, with an 18 per cent change in base sequence. There are some complications in using such information to produce evolutionary trees because of the strong tendency for purines to mutate to purines, and pyrimidines to pyrimidines²⁴. New statistical techniques can take this into account²⁵ by making a separate analysis of the evolutionary tree produced by each restriction enzyme and testing the statistical significance of each of these against that of the many others which can be generated from the same data. This new

mitochondrial genealogy of the primates (see the figure) helps to resolve several problems of human evolution^{25,26}. For example, many anthropologists claim that the chimpanzee, gorilla and human lineages diverged at the same time, and that their common ancestor used knuckles for walking. The new tree suggests that chimpanzees and gorillas separated about three million years ago, two million years after the divergence of the hominids. Their common ancestor was probably a small partially bipedal ape, and the chimpanzee-gorilla lineage lost the ability to walk upright which was retained in human evolution²⁷.

Mitochondrial genes may themselves be involved in the evolution of reproductive isolation. The success or failure of interspecific crosses often depends on which of the two acts as the female parent. Although this asymmetry can be caused by sex-linked loci²⁹, cytoplasmic genes may also be involved. For example, hybrid dysgenesis in *Drosophila* (which involves partial reproductive isolation between populations) results from an interaction between nuclear genes and inherited cytoplasmic factors²⁹. Analogous patterns of reproductive isolation among maize populations are known to result from mitochondrial genes for male sterility which are expressed only in conjunction with the appropriate nuclear genome³⁰.

Surprisingly enough, it has recently become clear that speciation can sometimes occur without interrupting the exchange of mitochondrial genes. Hybridization has long been a problem for plant systematics, and many amphidiploid plants have arisen by hybridization between two outcrossing ancestors. Information on nuclear and organelle DNA in amphidiploid *Brassica* and their parental species shows that while the derived species have obtained nuclear genes from both parents, their cytoplasmic DNA comes from only one³¹. A taxonomy based on mitochondrial DNA is not therefore the same as one derived from nuclear genes. In South American maize there are similar disagreements between classifications based on nuclear and on cytoplasmic genes which may be due to transfers of mitochondrial DNA across taxonomic barriers³².

Speciation in animals can also proceed without preventing the transfer of mitochondrial genes. Two species of mice, *Mus musculus* and *Mus domesticus*, meet in a narrow zone in Denmark. In this region hybridization allows nuclear genes from *domesticus* to penetrate for a few kilometres into the range of *musculus*. However, the extent of invasion by mitochondrial DNA is much greater, as in large parts of Scandinavia mice which can clearly be defined as *musculus* on the basis of their nuclear genes have a mitochondrial genome very similar to that of *domesticus*³³. Shared patterns of mitochondrial DNA are also found in some sympatric populations of *Drosophila pseudoobscura* and *D. persimilis*³⁴. In the

toadlets, *Bombina*, however, mitochondrial differences do not seem to stray through taxonomic barriers³⁴.

Mitochondrial DNA is able to penetrate the boundaries between species because it is not closely linked to genes responsible for maintaining reproductive isolation. Nuclear genes are embedded in chromosomes which contain loci subject to strong selection. This makes introgression much more difficult, as the flux of genes through a hybrid zone depends directly on the ratio of fitnesses of hybrid and parental populations, but exponentially on the harmonic mean recombination rate between the introgressing loci and those subject to selection^{36,37}. Small changes in linkage can therefore have drastic effects on gene flow: for example, if hybrids are one fifth as fit as their parental populations then mitochondrial genes (with a recombination rate of about 0.5) will flow through a hybrid zone about 15,000 times faster than a typical nuclear gene with a harmonic mean recombination rate of, say, 0.2.

The ability of mitochondrial DNA to pass through the barriers between taxa may mean that it is sometimes necessary to

define species only in terms of nuclear genes. Since rates of gene flow vary with the strength of selection on surrounding regions of chromosome, the degree of reproductive isolation (and the assessment of taxonomic affinity) may sometimes vary from locus to locus even within the nuclear genome. It may also lead to ambiguities in the interpretations of patterns of relatedness based on mitochondrial genes: if hybridization has led to the invasion of one evolving lineage by the mitochondrial genes of another, both the extent of their genetic divergence and the time which has elapsed since separation will be underestimated.

The degree to which this may obscure the evolutionary history of diverging populations is not yet known, so that it is still necessary to treat patterns of relatedness based on mitochondrial DNA with a certain caution. For example, if it is assumed that mitochondrial evolution has proceeded independently and at the same rate within all human populations, then a simple 'molecular clock' of divergence suggests that African Bushmen separated from other human lineages about 220,000 years ago, while the split between Asians and Europeans was only 5,500 years before the present²³.

This disagrees both with the fossil evidence and with estimates of differentiation based on nuclear genes. There must therefore have been differences in the rate of divergence in the mitochondrial genomes of various human populations, perhaps because of variations in the extent to which mitochondrial DNA has crossed the barriers between the evolving groups. Bushmen and Australian Aborigines may have been relatively isolated from invasion by other mitochondrial genomes and may hence have accumulated genetic changes more rapidly than did other populations.

In spite of these problems, mitochondrial DNA has already provided remarkable insights into the evolutionary history of animals and plants. An analogy may be drawn with human surnames, which have a high mutation rate, are inherited through one parent and evolve rapidly. The distribution of markers such as Jones, Schmidt or Dupont throughout Europe gives a much more vivid picture of recent population history than does, for example, that of the ABO blood groups. This means that the mitochondrial genome is particularly valuable for studying patterns of divergence between closely related populations. Its value in understanding human social history is less clear. The matrilineal patterns of mitochondrial inheritance do mean that we are all more closely related to our mothers than to our fathers; to Eve than to Adam. What will sociobiology make of this? □

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Carbon isotope data in core V19-30 confirm reduced carbon dioxide concentration in the ice age atmosphere

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Precise and detailed measurements have been made of the ^{13}C content of both planktonic and benthic foraminifera in core V19-30. Using the approach developed by Broecker, a record of atmospheric CO_2 concentration during the past 120,000 years has been derived which provides independent confirmation that the CO_2 concentration in the glacial-age atmosphere was much lower than it is today.

THE concentration of carbon dioxide in the atmosphere is controlled in a complicated manner by the dissolved carbon dioxide content of ocean surface water (complications arise chiefly because the large temperature range over the ocean surface gives rise to a considerable range in CO_2 partial pressure, so that CO_2 is absorbed at high latitudes and released at low latitudes¹). Life in the ocean has a marked effect on atmospheric CO_2 ; the CO_2 content of surface water is lower than that of deep water as a result of the continual removal of carbon from the surface by photosynthesis. Since photosynthesis preferentially removes isotopically light carbon from the surface carbon pool, ^{13}C variations in the ocean reflect this process. Starting from this observation, Broecker² attempted to model the low carbon dioxide content of the last glacial as reported by Delmas *et al.*³ and by Neftel *et al.*⁴ in their analysis of air bubbles in polar ice cores.

The steady-state partial pressure of carbon dioxide in the atmosphere depends on the total CO_2 content of ocean surface water and on its temperature, salinity and alkalinity. Broecker² calculated the effects of glacial-interglacial changes in ocean surface temperature and salinity and found that together they could account for only a 10 p.p.m.v. (parts per million by volume) change in atmospheric CO_2 ; he argued that glacial-interglacial changes in alkalinity could be neglected. However, the CO_2 content of surface water is kept below that of deep water by the photosynthetic removal of carbon, to a degree that is controlled by the nutrient content of the ocean. Broecker² reasoned that a higher ratio of phosphorus (taken as the limiting nutrient) to carbon in the glacial ocean would entail enhanced removal of organic carbon and thus a reduction in the CO_2 partial pressure. Broecker proposed that during the sea-level rise following the last glaciation, phosphate was removed from the ocean together with organic carbon in the sediments that accumulated on the continental shelves as these were flooded.

Since marine photosynthesis results in a ^{13}C fractionation of about 23‰, Broecker's mechanism² would associate a higher oceanic phosphorus concentration with an increased difference between the ^{13}C content of surface and deep water dissolved CO_2 and a diminished atmospheric CO_2 content. Broecker² supported his model by summarizing the best available ^{13}C data from Holocene and last glacial foraminifera. Here we have made detailed ^{13}C measurements with a view to deriving a continuous record of the changing vertical ^{13}C contrast.

Data

We chose to study core V19-30, which was raised from a water depth of 3,091 m at 3°23' S, 83°21' W, on the Carnegie Ridge

to the south of the Panama Basin. Earlier work⁵ showed that cores from this area are capable of yielding excellent oxygen isotope records. We sampled the core at approximately 3-cm intervals; data for the upper 800 cm, covering about 130 kyr, are shown here. Samples were disaggregated in distilled water and foraminifera picked from the sand fraction after sieving. Measurements of the isotopic composition of carbon dioxide released from the carbonate foraminifera were made using a VG Isogas triple collector mass spectrometer. The deep water record is obtained from *Uvigerina senticoso*; about eight specimens were used for each analysis.

A major reason for the success of stable isotope studies in this region is that there has evidently been a good population of this species throughout the past few hundred thousand years⁶; in other areas it is frequently necessary to piece together a record using different species for different sections⁵. It is more difficult to obtain a good surface water record in the region because varying seafloor dissolution has removed the more solution-susceptible species during parts of the time interval studied; we chose to analyse the relatively dissolution-resistant *Neogloboquadrina dutertrei*. This species has recently been shown to calcify at about 30 m depth, in water of the same temperature throughout the year, in the region of our study⁷. To minimize the effect of ontogenetic development on the isotopic composition of the test⁸, specimens were selected from a controlled size range, 355–450 μm . Where possible (~70% of the samples), more than 30 specimens were used; the use of this relatively large number also reduces analytical noise. Unfortunately, several samples near the top of the core contained insufficient planktonic specimens for analysis.

Figure 1 shows the oxygen isotope record for *U. senticoso*, which provides a standard stratigraphy for the core^{9,10}, together with the carbon isotope data for both *N. dutertrei* and *U. senticoso* (the oxygen isotope data for *N. dutertrei* will be discussed elsewhere). The carbon isotope data for *Uvigerina* have been adjusted by 0.9‰ so as to provide our estimate of the actual ^{13}C content of dissolved CO_2 in the water from which the tests were calcified¹¹. No equivalent adjustment factors have been determined for planktonic species; however, the down-core record must reflect the pattern of change in the ^{13}C content of near-surface water provided there are no unknown factors causing change in either the degree of departure from ^{13}C equilibrium or the calcification depth of the specimens available for analysis.

To facilitate discussion, the data in Fig. 1 have been plotted on an approximate time scale rather than a depth scale; this was obtained by the now-standard method of correlating

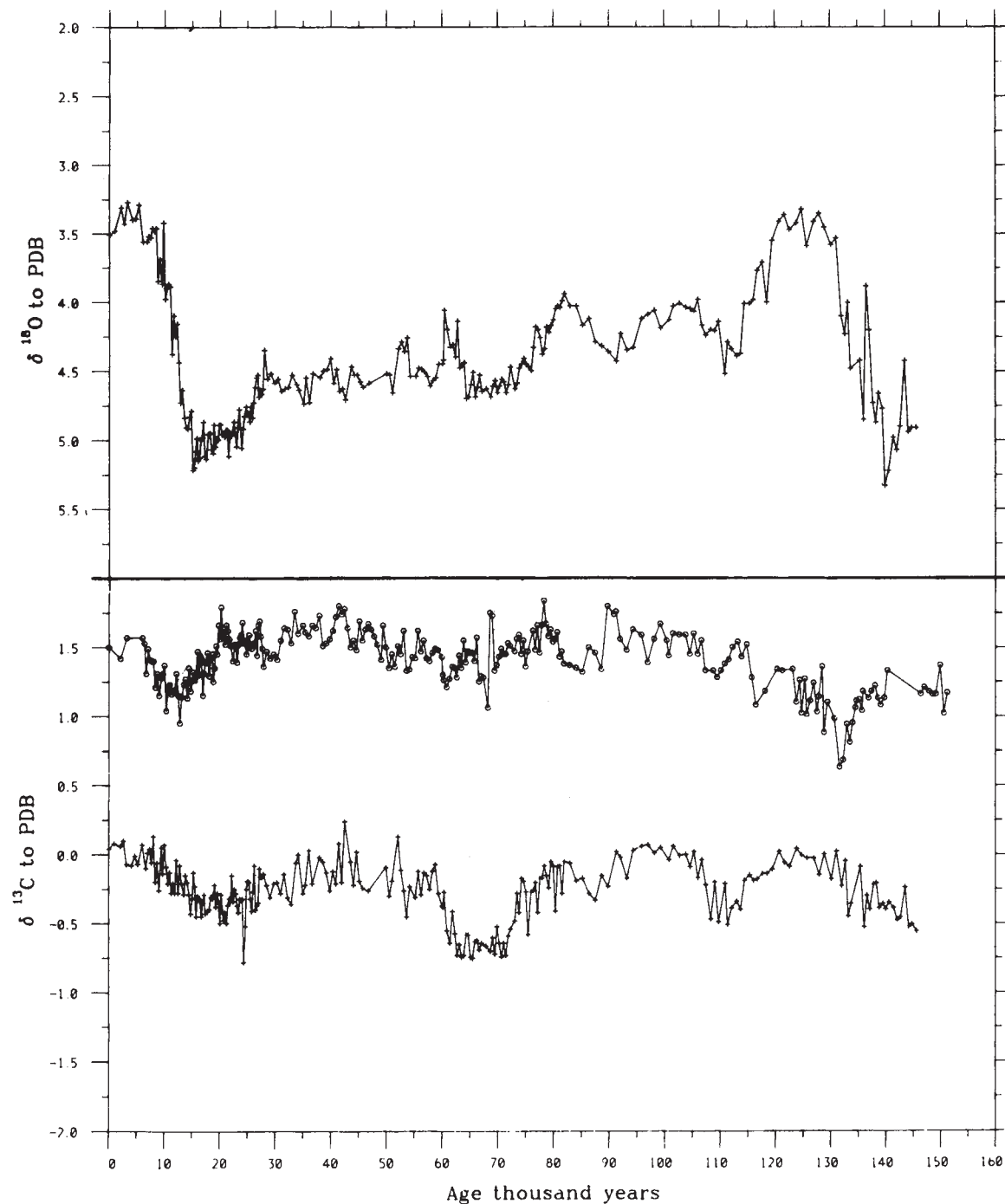


Fig. 1 Top, oxygen isotope data for *Uvigerina senticosus* in V19-30. Below: carbon isotope data for *Neogloboquadrina dutertrei* (○) and for *U. senticosus* (+). The data are plotted on a simple time scale derived from ^{14}C data and by correlation with terraces in New Guinea for which radiometric dates are available¹².

features in the ^{18}O record in this and other cores with coral terraces dated by the ^{230}Th and ^{231}Pa methods¹²; we have used some ^{14}C control in the upper part (the exact time scale used is immaterial to the present discussion).

It is clear that the two carbon isotope records in Fig. 1 are not identical. As Broecker² has pointed out, the ^{13}C records for surface and deep water would be identical if Shackleton's hypothesis⁵ were a complete explanation for the data. Shackleton⁵ proposed that the transfer of isotopically light carbon between the ocean and the terrestrial biomass was the explanation for the marked ^{13}C variations which he discovered. It is now known that a portion of the variation that he found in the deep Atlantic reflects oceanographic changes in that ocean¹³ and that the Pacific is more likely to yield a representative ^{13}C record for the global ocean. Thus, the ^{13}C record of

benthic foraminifera shown in Fig. 1 may reflect changes either in the terrestrial biomass (as Shackleton⁵ proposed for the larger variations that he found in the Atlantic), or in the organic matter stored on the continental shelves as Broecker² suggested, or both. The obvious changes in the surface-to-deep water ^{13}C difference cannot derive solely from the changing partitioning of carbon between the ocean and either of these reservoirs of isotopically light carbon. However, Broecker² points out that storage in carbon-rich sediments on the continental shelf would also involve phosphorus, so providing a mechanism for changing the phosphorus content of the ocean.

Broecker² concluded from the data available that the mean planktonic ^{13}C values for glacial and for Holocene times differed by only 0.1‰ (but with considerable scatter); he therefore reasoned that the difference between glacial and Holocene

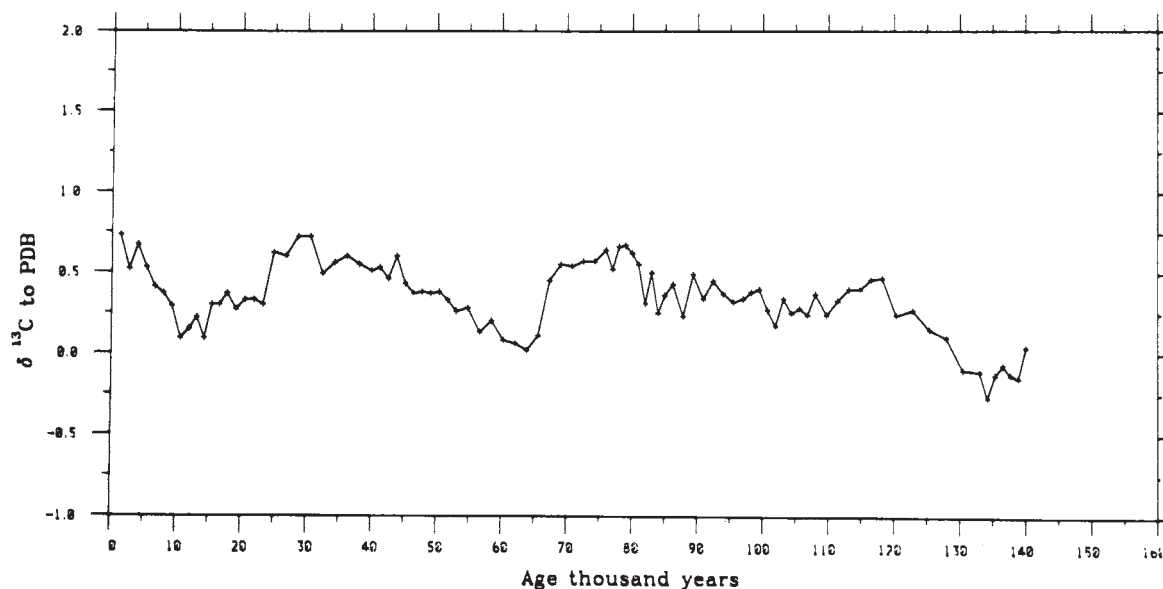


Fig. 2 Carbon isotope data for *Globigerina bulloides* (surface water) in core RC11-120, plotted on an equivalent time scale to Fig. 1 derived by correlating the oxygen isotope record of core RC11-120 with that of V19-30.

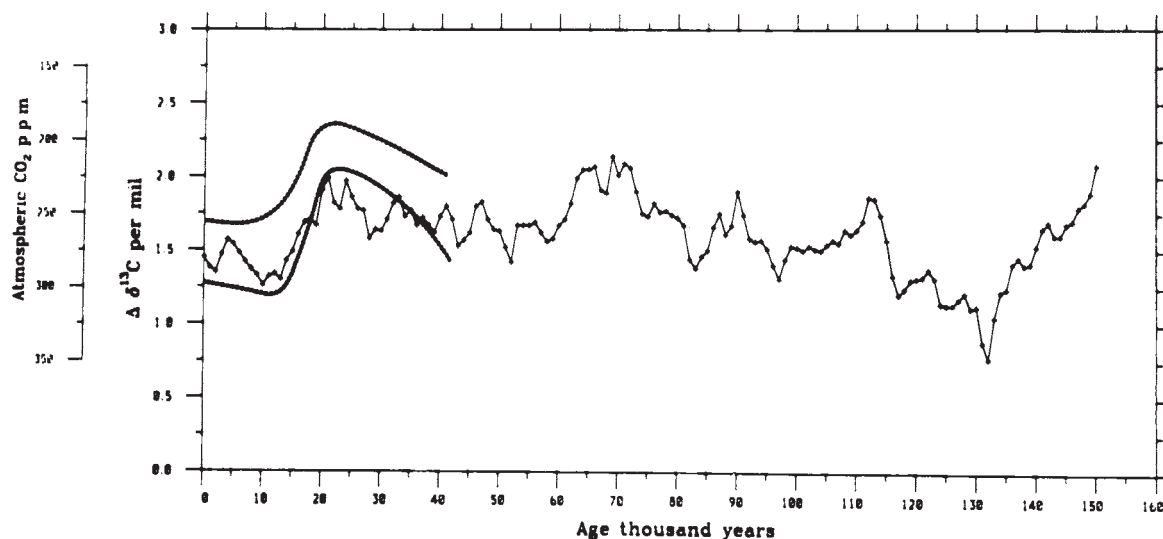


Fig. 3 Carbon isotope difference between ocean surface (*N. dutertrei*) and sea floor (*U. senticosus*) in core V19-30. A scale of equivalent level of atmospheric CO₂ content is constructed assuming that the measurements at the top of the core correspond to a pre-industrial CO₂ content of 280 p.p.m., and using Broecker's model² to scale the isotope range. The heavy lines delineate the limits between which Neftel *et al.*⁴ estimated as the range.

benthic ¹³C values would provide an estimate of the changing surface-to-deep difference. Inspection of Fig. 1 suggests that this simple model is not correct in detail; the range of variation in surface-water ¹³C is similar to that in deep water, so that it is necessary to make a subtraction in order to predict atmospheric CO₂ content. However, before discussing the subtraction it is important to establish a case for regarding these particular data as typifying the carbon isotope record of warm oceanic surface and of globally averaged deep waters.

As regards the deep water record, the situation is complicated by the fact that the production of North Atlantic deep water changed dramatically with Pleistocene climatic change, so that in the Atlantic there is a larger ¹³C excursion than in the Indian and Pacific Oceans¹³. It might be thought that under the region of high productivity in the eastern equatorial Pacific the deep water would show an additional ¹³C effect associated with enhanced surface productivity during glacials¹⁴, but our unpublished data show a similar (although less detailed) ¹³C record in the deep water of the western Pacific. Thus, the present record

is probably a reasonable approximation to a global average, and has the advantage of better accuracy and stratigraphic resolution than any other published ¹³C records (the true global change in deep-water ¹³C content may have been greater by of the order of 20‰ in view of the above-mentioned complex events in the North Atlantic).

As far as surface waters are concerned, the chief problem has been the poor signal-to-noise ratio generally obtainable. Figure 2 shows the result of a re-analysis of core RC11-120¹⁵, in which triplicate analyses of *Globigerina bulloides* from the 355–450-μm range were made at 5-cm intervals. Core RC11-120 was raised from 3,135 m at 43°31' S, 79°52' E in the sub-Antarctic Indian Ocean. The ¹³C data are plotted in Fig. 2 on a time scale designed to correlate the ¹⁸O record of the core with that shown in Fig. 1 for V19-30.

The ¹³C data in Fig. 2 show a similar pattern of variation to that shown by *N. dutertrei* in Fig. 1; they show no relationship to the estimated temperature record for the core¹⁵, and are thus unlikely to result from changing local factors. The differences

between the records of V19-30 and RC11-120 are sufficiently subtle that considerably more work will be needed to establish whether they are chiefly due to time-scale uncertainties, analytical errors or real differences in the ^{13}C histories of the two regions. For the present we assume that both records do indeed approximate the history of average sea-surface ^{13}C variations. Broecker², with access only to low-resolution records, simply concluded that the glacial ^{13}C values were only about 0.1‰ different from recent values. Although our new data do not conflict with this generalization, it is apparent that high resolution measurements reveal an important signal whose amplitude is similar to that in benthic foraminifera. It is unfortunate that in order to obtain a high-quality stratigraphic record one is obliged to work in an area of high productivity, since the experiment would ideally be performed in a mid-gyre area in order to minimize the possible effect of varying upwelling rates. In areas of intense upwelling the surface water is renewed from below so fast that photosynthesis is not nutrient-limited, so that ^{13}C is not a measure of the phosphorus content of the upwelling deep water. Although it will not be possible to repeat this experiment in such an area, we plan to verify the general nature of the planktonic ^{13}C record by careful analysis of mid-gyre cores. Since the V19-30 planktonic record is the most accurate and detailed that we have obtained, as well as having the incomparable advantage of being derived from the same core as the benthic record (eliminating any correlation problems), no case can be made at present for attempting to obtain a more general record by incorporating available data from other cores. We proceed on the assumption that the surface-water and deep-water ^{13}C records shown in Fig. 1 are representative of global variations in these quantities.

Figure 3 shows the surface-to-deep water ^{13}C difference as a function of estimated time. The data are averaged over 1-kyr intervals to reduce the effect of random analytical uncertainty and to remove possible complications arising from the fact that ocean mixing takes several hundred years. The ^{13}C record is also shown with a scale for changes in atmospheric carbon dioxide content, calculated (after Broecker²) from the implied record of surface-water CO_2 content. The scale is positioned on the assumption that the ^{13}C difference observed at the top of the core reflects a pre-anthropogenic atmospheric CO_2 level of about 280 p.p.m.v. The positioning of the scale is poorly constrained because of our inability to obtain a detailed record from *N. duterrei* in the uppermost part of the core. Broecker also estimated the likely effect of changing ocean salinity (due to the removal of fresh water to the ice sheets) and of surface temperature. To a first approximation these may be regarded as proportional to the ^{18}O record; the total range associated with the first is about 12 p.p.m. (more CO_2 in the glacial) and the second 20 p.p.m. (less CO_2 in the glacial), so that together they would contribute less than 10 p.p.m.

Discussion

Figure 3 also indicates the range within which Neftel *et al.*⁴ estimated past CO_2 concentration to have varied. The range that we have calculated is entirely consistent with their data. Our record even shows a slight diminution in CO_2 concentration during the Holocene, and near-Holocene values about 40 kyr ago, as found by Neftel *et al.* More important, the timing of the transition is in the form of a rapid rise in CO_2 content centred precisely on the deglaciation. If one were to assume, as Broecker² was forced to do, that the ^{13}C variations in surface water could be neglected, the form of the transition would appear more gradual, and less consistent with the ice-core data⁴.

Beyond the time range covered by the ice-core data⁴, it is interesting that during the interglacial interval of substage 5e, indicated between 130 and 120 kyr on the age model used here, the atmospheric CO_2 level was higher than the pre-anthropogenic Holocene level. It is generally believed that global temperatures were slightly higher during 5e than during the Holocene and indeed several workers have proposed 5e as a geological scenario for our high- CO_2 future. This enhanced CO_2 level persisted during the early stage of glacial advance between 120 and 116 kyr.

More work will be needed to elucidate the cause of the ocean chemical changes documented here. A valuable contribution has been made by Keir and Berger¹⁶, who made a numerical model to reconstruct changes in atmospheric CO_2 concentration. They assumed that Broecker's model² was appropriate, and that storage of carbon and phosphate on the continental shelves could be regarded as controlled by sea-level rise and fall, and so by the derivative of the ^{18}O record. The carbon isotope data shown here are essential constraints for any future model of this nature.

Finally, we note that the important measurements made in polar ice cores^{3,4} were technically difficult, so that when Broecker² discussed his model he admitted the possibility that their observations would not be supported by further investigations. Thus, we consider that a record of climatically significant changes in the carbon dioxide content of the atmosphere during the geologically recent past, derived by a totally independent method, is of considerable significance. Moreover, our method has the great advantage that it will be possible to produce a high-resolution record covering several glacial-interglacial cycles, and so better to understand the mechanism by which the well established orbital variations¹⁵ incorporate changing carbon dioxide levels in the feedback loop through which they control climate.

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Significance of ferrodioritic liquids in magma mixing processes

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A general model to explain the various igneous associations found in mixed-magma intrusives and extrusives belonging to high-level volcanic centres is presented. This model is based on detailed mapping in the British Tertiary Volcanic Centre on Skye which suggests that basic and acid magmas were continually available throughout its development. New major- and trace-element data from these rocks indicate that they were co-genetic, and that the basic magmas which combined with acid magmas to produce hybrid magmas were ferrodioritic in composition, and not basaltic as had previously been thought.

THE various extrusive and intrusive rocks of the British Tertiary Volcanic Province (BTVP) of north-west Scotland and north-east Ireland have been the subject of numerous investigations over the past 200 yr. Eight discrete centres of activity are at present exposed above sea level, whilst at least three submerged centres have also been recognized. They are all located within continental crust and the main intrusive units of each of the centres have an average age of ~59 Myr. Distinctly acidic and basic rock-types constitute the dominant components of these centres¹⁻⁵, whereas rocks of intermediate composition which have developed by fractional crystallization processes are relatively uncommon⁶.

However, several examples of rocks which have intermediate compositions do occur, although it has been concluded from field, mineralogical and geochemical evidence that they are the products of processes involving the intimate mixing or comingling of acidic (granitic) and basic (s.l.) magmas^{7,8}. This article discusses the nature of the various magmas involved in these examples of magma mixing in the light of new chemical data from the Skye centre and presents a model for their evolution in the subvolcanic chambers which existed below BTVP and similar centres, which exhibit distinctly bimodal magma compositions.

Evidence for magma mixing

Features common throughout the BTVP include:

- (1) Composite sills and dykes, which have basic margins and acid centres. Detailed investigations of several of these minor intrusions⁹⁻¹² indicate that the basic magma was introduced first, and that after a short period of time the acid magma followed. Chilling of the hotter, basic magma against both the country-rock and the cooler acid magma is readily evident¹³. Along the interface between the acid and basic units there is abundant evidence for both mechanical mixing and *in situ*, diffusion-controlled hybridization^{9,14}.
- (2) Net-veined complexes, within which there have been numerous phases of interaction between acid and basic magmas, often with the former severely veining the latter¹⁵⁻²⁰. Such mixed intrusive associations are typically composed of brecciated or pillow-shaped masses of basic material which are enclosed in an acid host (such as granophyre or felsite). Where diffusion-controlled hybridization has not occurred to any great extent, chilling of the basic pillows against the granophyre or felsite is commonly seen^{13,19,20}.
- (3) Ring-dyke intrusions, which form an extremely varied group, and are composed of material ranging from predominantly acid compositions containing entrained blebs and streaks of wispy basic material (such as in the Loch Ba Felsite, Mull and the

Glamaig Epigranite, Skye²¹), through to completely mixed rocks of intermediate composition and colour (such as in the marscoite ring-dyke, Skye⁷). Between these two extremes there are numerous examples of ring-dykes of hybrid material which are far from homogeneous, but which do show good evidence that intimate mixing of acid and basic magmas has taken place (for example, glamaigite from the Marscoite Suite of Skye⁷, and the Kilchrist Ring-dyke of Skye¹⁴).

Although distinct categories of acid-basic relationships have been presented above, it should be noted that several examples exist which show features belonging to more than one of these groupings. For example, in the composite sill suite of the Eastern Red Hills district of the Skye Centre there are cases where fragments of basic material are enclosed in the central felsite units⁹. Possible causes for the large variation in the mutual relationships exhibited between the acid and basic components in each of these associations will be considered below.

In all of the examples listed above various petrographic features indicate that mixing of two (or in some cases more than two) discrete magmas has taken place. The hybrid liquids which developed had compositions which were not in equilibrium with the phenocryst assemblages of either the original acid or basic parents. Hence, we see frozen-in disequilibria phenomena, such as: (1) Quartz crystals surrounded by layers of pyroxene and amphibole in a corona arrangement, (2) olivine which has been partially replaced by pyroxene, (3) non-mechanically rounded plagioclases, (4) alkali feldspars exhibiting the so-called fingerprint texture, indicating that they were reacting with and dissolving in the surrounding magma before its solidification.

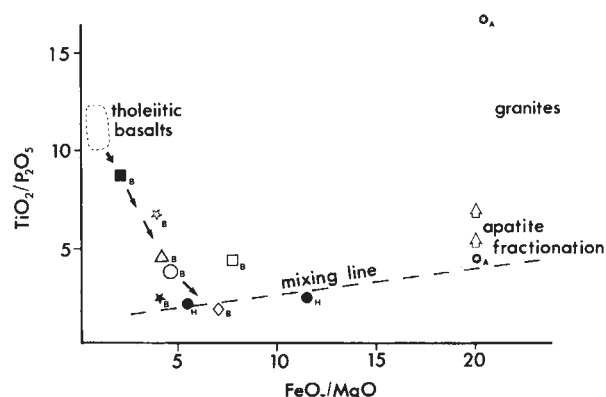


Fig. 1 Plot of $\text{TiO}_2/\text{P}_2\text{O}_5$ versus FeO_7/MgO . B, H and A are basic, hybrid and acid rocks of mixed-rock associations (■, B31: base; ☆, MS376; △, SK708; □, MS201X; ○, H4540; ★, B95; ◇, H344; ●, H869 and H871; ◊, H4351 and H868—see Table 1); area outlined by dashed line is field of typical BTVP tholeiitic basalts^{22,23,40}. Data for SK708 from ref. 21.

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Table 1 Major- and trace-element data for basic, acid and hybrid members of mixed rock associations

	B31: base	B31:230	H344	H868	H4540	H4351	H871	H569	SK708	MS201X	MS376	B95
SiO ₂	55.25	69.73	52.93	75.93	48.87	68.59	59.17	56.71	59.94	58.37	51.86	51.36
TiO ₂	1.05	0.56	2.35	0.16	3.08	0.63	1.46	1.97	1.46	1.69	2.06	2.06
Al ₂ O ₃	14.42	13.28	12.69	11.72	12.83	13.02	13.27	12.43	13.45	13.27	13.03	13.43
FeO _T	9.77	5.04	14.24	2.10	15.64	5.83	11.10	11.09	9.47	12.56	14.61	12.28
MgO	4.74	0.72	2.02	0.01	3.48	0.29	0.97	1.96	2.30	1.61	3.69	3.11
MnO	0.12	0.06	0.30	0.03	0.28	0.17	0.26	0.24	0.17	0.23	0.23	0.27
CaO	7.29	0.72	6.23	0.37	7.56	1.96	4.68	4.76	4.80	5.72	7.14	5.83
Na ₂ O	2.62	4.31	3.43	3.52	3.10	3.83	3.82	3.70	4.54	3.72	4.51	1.67
K ₂ O	1.90	3.49	1.91	5.12	1.25	4.00	2.85	2.66	0.59	1.80	1.49	3.34
Total alkalis	4.52	7.80	5.34	8.64	4.35	7.83	6.67	6.36	5.09	5.52	6.00	5.01
P ₂ O ₅	0.12	0.10	1.18	0.01	0.77	0.14	0.60	0.87	0.32	0.37	0.30	0.83
H ₂ O ⁻	2.39	1.49	0.26	0.12	0.46	0.43	0.06	0.23	—	0.07	0.15	4.42
LOI	0.30	0.19	1.40	0.50	2.25	0.81	0.67	2.33	2.90	0.20	0.05	0.78
Total	99.98	99.69	98.94	99.59	99.57	99.70	98.91	98.95	99.94	99.60	99.13	99.39
Ba	658	897	1646	363	803	1795	3096	1253	—	537	342	1765
Rb	56	82	39	164	30	112	56	66	—	61	65	76
Sr	163	63	260	9	207	138	186	186	—	158	176	263
Nb	19	41	21	31	18	16	17	22	—	21	21	22
Zr	343	732	406	431	193	408	557	302	—	278	199	439
Y	53	102	79	58	59	67	69	70	—	64	93	55
Th	4	7	2	7	3	6	3	3	—	7	5	3
Cu	78	18	49	8	22	9	89	61	—	30	47	23
Ni	54	5	6	5	32	8	8	7	—	10	46	31
V	223	36	84	3	376	12	52	111	—	209	339	188
Zn	86	106	139	46	134	102	125	136	—	107	176	121
Cr	105	19	11	11	18	8	9	8	—	14	48	32
La	21.0	48.5	41.7	106.7	29.2	48.7	40.2	44.3	—	—	—	—
Ce	53.4	100.6	92.9	199.0	62.9	106.6	92.0	98.0	—	—	—	—
Pr	4.7	12.9	12.5	25.0	8.5	12.6	11.5	12.3	—	—	—	—
Nd	24.9	53.8	57.2	97.1	38.2	52.1	51.8	53.5	—	—	—	—
Sm	6.2	11.4	11.9	16.8	8.3	10.2	10.3	10.9	—	—	—	—
Eu	1.62	2.41	5.03	1.04	2.97	3.08	5.12	3.81	—	—	—	—
Gd	6.6	11.8	12.38	13.0	8.8	10.2	10.4	11.0	—	—	—	—
Dy	7.63	13.17	11.63	12.04	8.64	10.61	10.16	10.54	—	—	—	—
Ho	1.58	2.77	2.29	2.28	1.72	2.22	2.06	2.11	—	—	—	—
Er	4.83	7.45	5.87	5.78	4.45	6.10	5.38	5.46	—	—	—	—
Yb	4.56	8.09	5.62	5.84	4.28	6.85	5.53	5.45	—	—	—	—
Lu	0.71	1.20	0.83	0.82	0.63	1.05	0.86	0.81	—	—	—	—

B31: base, basic margin of the Rubh' an Eireannaich composite sill, Skye; B31:230, acid centre of the Rubh' an Eireannaich composite sill, Skye; H344, ferrodiorite from the Marscoite Suite, Skye; H868, Southern Porphyritic Felsite from the Marscoite Suite, Skye; H4540, basic inclusion in the Marscoite Suite, Skye; H4351, Meall Buidhe Epigranite, Skye; H871 and H569, marscoite, Skye; B95, basic member of basic-acid (mixed) lava, Kilchrist, Skye; SK708, basic inclusion from the Glamaig Epigranite, Skye²¹; MS201X, basic inclusion from the Loch Ba Felsite, Mull; MS376, basic material from the Beinn a' Ghraig net-veined complex, Mull. Major-element oxides are given in weight % and trace-elements in p.p.m.

Typically the groundmass in each of the examples given above is relatively homogeneous, although in some products of magma mixing reported elsewhere this is not so readily evident²¹.

Whole-rock geochemistry

New whole-rock compositional data for basic, acid and mixed rocks from the Skye Centre (plus two analyses of basic components from the Mull Centre) are listed in Table 1.

Several of the so-called dolerites and basalts which constitute the basic components have evolved compositions, with SiO₂ > 51%, Na₂O + K₂O > 4% and FeO_T/MgO > 3. These values, when compared with data from spatially and genetically associated tholeiitic basalt lavas from the BTVP (see below)^{22,23} indicate that the basic magmas involved in occurrences of acid-basic mixing had reached a stage of extreme alkali- and Fe-enrichment.

When compared with the less-fractionated tholeiites, the concentrations of certain minor-elements recorded from the basic components of these mixed rock associations are significantly different, with TiO₂ > 1.5% (1.0–1.5%) and P₂O₅ > 0.3% (<0.15%) (with typical BTVP tholeiitic basalt data from ref. 24 in parentheses). Furthermore, distinctive values of TiO₂/P₂O₅ illustrate the evolved nature of these rocks when plotted against FeO_T/MgO (Fig. 1).

A distinct trend from primitive tholeiitic compositions towards Fe-enriched compositions is present within the basic components of the mixed-rock associations of the Skye Centre. In particular, the TiO₂/P₂O₅ ratio recorded from the ferrodiorite ring-dyke (H344, see Table 1) of the Marscoite (mixed-rock) Suite is significant, as it may be concluded that its composition could only have been derived by ~95% fractional crystallization of a more primitive tholeiitic basalt^{25,26}. The least evolved specimen reported here (B31: base, Table 1) indicates that ~50% crystal fractionation of a typical tholeiitic basalt parent must have occurred in order to derive its composition.

Data for the rare-earth elements (REE) have been obtained from various basic, acid and hybrid rocks belonging to acid-basic associations (Table 1).

In the first instance it is useful to compare the respective chondrite-normalized REE patterns from the basic margins and acid centre of a typical composite sill. As illustrated by Fig. 2, the patterns are parallel, and the acid centre shows an overall enrichment in REE when compared with the basic margin. These two observations suggest that: (1) as evidenced by their parallel patterns, the basic and acid magmas are co-genetic; (2) the two magmas did not evolve from a homogeneous silicate melt due to the effects of liquid immiscibility. If this were the case, then, as deduced from various experimental studies^{27,28}, the basic

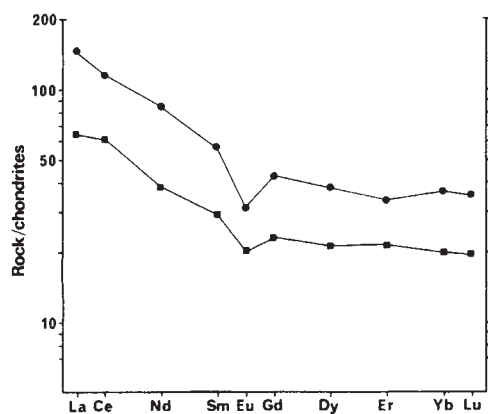


Fig. 2 Chondrite-normalized⁴¹ REE plots for the basic margin (■) and acid centre (●) of the Rubh' an Eireannaich composite sill, Skye.

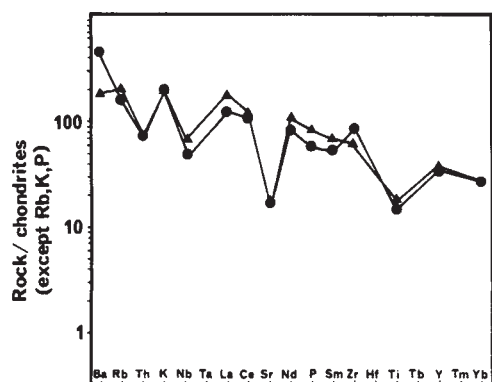


Fig. 3 Normalized incompatible-element plot²⁴ for marscoite from the Western Red Hills, Skye, and a theoretical mix calculated from postulated basic and acid compositions (found in close association) by a least-squares technique using major-element data^{42,43}. ●, Marscoite (H871) from the southern part of the Western Red Hills; ■, theoretical mix based on least-squares estimate of basic and acid components, using 73.82% of H344 (ferrodiorite) and 26.18% of H868 (Southern Porphyritic Felsite).

component would show an enrichment in the REE over the acid component by a factor of between 5 and 10. This is clearly not the case in this example, and has not been recorded from other similar acid–basic associations in the BTVP.

Mixing relationships of hybrids

The general field relationships of certain acid–basic associations indicate that the mixing processes have, for various thermal and mechanical reasons, not gone to completion. However, certain hybrid rocks in the BTVP show a relatively high degree of homogeneity and it is only when one examines carefully their mineralogies that evidence of mixing is found in the form of xenocryst assemblages.

One of the most famous examples of the latter type which is found in the Skye Centre is the rock-type which is referred to as marscoite⁹. Wager *et al.*⁷ made a detailed study of marscoite and other closely related rocks, including the ferrodiorite ring-dyke discussed above (H334). They also investigated the associated acid rocks (granites and felsites) in an attempt to identify the acid and basic parents which mixed to produce marscoite. On the basis of their close field relationships and phenocryst assemblages the basic parent was identified as ferrodiorite, whilst the acid parent was concluded to be the so-called Southern Porphyritic Felsite (SPF) (H868, Table 1).

For the present study these two parental compositions were chosen and their major-element compositions were put in a least-squares mixing procedure and compared with an analysis of marscoite (H871). A small residual sum of squares (1.1941) and hence good statistical fit was obtained. This is graphically illustrated by the mixing line in Fig. 1.

Although this does not prove that the identified parents are the correct ones, such a calculation would indicate if they were definitely incorrect. Values obtained by this approach are:

$$73.8\% \text{ ferrodiorite} + 26.2\% \text{ SPF} = \text{marscoite}$$

Taking these proportions and considering the new trace-element data (Table 1) it is possible to represent the hypothetical mix and the marscoite in terms of a normalized incompatible-element plot and to compare their respective patterns (Fig. 3). A very good approximation is obtained when the percentages generated in the least-squares method outlined above are used.

In contrast, the mixing of acid and basic magmas in the composite sill and dyke association appears to have proceeded to a far lesser extent. Typically the basic magma chills against the later, cooler acid component, and there is a limited amount of mechanical mixing. However, Harker⁹ recognized that the Rubh' an Eireannaich sill of the Eastern Red Hills Suite of Skye no definite contact existed between the two contrasting rock-types. Thus, one passes from the basic margins (for example, B31: base, Table 1) of the sill into the acid centre (B31:230) without crossing a sharp compositional break. All other composite sills and dykes in this suite show distinctive boundaries between the acid and basic components. This significant feature of the Rubh' an Eireannaich sill is interpreted here in terms of diffusion at high temperature, a component of which may have occurred after the intrusion of the magmas to their present level in the Earth's crust. Evidence for a small amount of mechanical mixing before the intrusion of these magmas into their present position can be recognized by the presence of alkali feldspar xenocrysts in the basic component (which occur as phenocrysts in the felsite unit).

To investigate further the compositional nature of this sill a series of samples was collected from throughout its thickness. Figure 4 presents major and trace-element profiles through the sill, from which it can be readily appreciated that all of the elements determined show relatively continuous variation patterns. Irregularities do occur and are interpreted as being the result of alteration processes caused by hydrothermal systems set up when the nearby granites and gabbros were intruded²⁹.

In total the sill is 5 m thick, with ~1.3 m of basic material flanking the central acid unit on each side. As can be seen from Fig. 4, steep major- and trace-element compositional gradients exist in the sill profile at 1.3 m in from both margins. Differences in gradients for various elements are interpreted in terms of the relative mobility of the species involved¹⁹. For example, the gradients shown by highly incompatible elements such as Ba, Rb, K and Na tend to have much shallower profiles due to the fact that they have been able to diffuse to a greater extent and have approached equilibrium over greater distances than some of the more compatible elements (for example, Ni, Cu, Mg, Cr and Fe).

All the features outlined above indicate that the intimate mixing of two contrasting magmas depends not only on thermal constraints (for example, the percentage of phenocrysts in each of the magmas), but also on the mechanism by which the two magmas came together and mixed. We are seeing, therefore, evidence of an extremely broad spectrum of different magma mixing processes within the Skye Centre and, in general, the BTVP. This ranges from composite sills and dykes at the almost 'no mixing' end, through associations such as net-veined complexes and granitic ring-dykes which contain wisps and streaks of basic material disseminated throughout their mass, to ring-dyke intrusions of the marscoite-type which show a high degree of homogeneity.

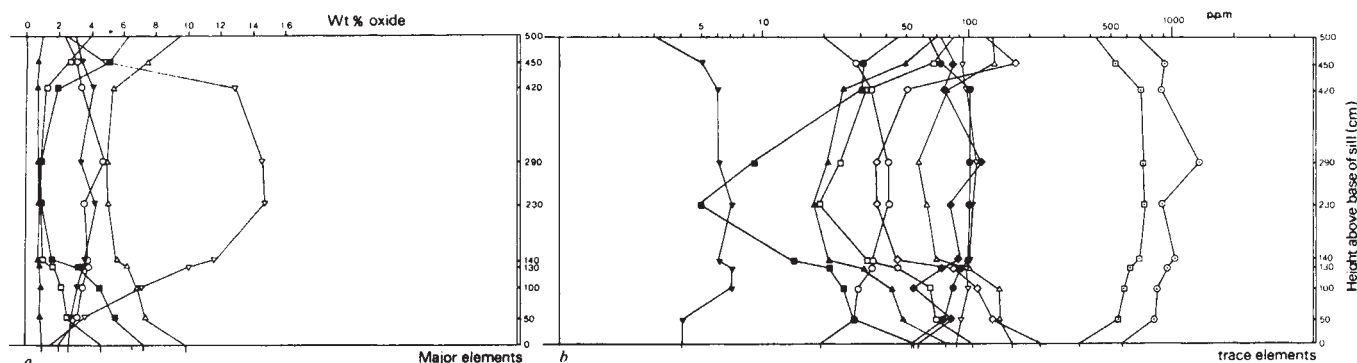


Fig. 4 Variation diagram for: *a*, major-element oxides (▲, TiO_2 ; □, MgO ; ▼, Na_2O ; ○, K_2O ; ▽, SiO_2 —55%; ■, CaO ; △, FeO_7); *b*, trace elements (▼, Th; ■, Ni; ○, Nb; ▲, Cu; ◆, Rb; □, Cr; ●, Y; ▽, Zn; ◇, V; △, Sr; □, Zr; ○, Ba) in a traverse through the Rubh' an Eireannaich composite sill, Skye.

Magma interaction processes

In recent years numerous field and laboratory investigations have been made in an attempt to understand the mechanisms of fractionation which are thought to occur in subvolcanic chambers^{30–35}. Such studies suggest that it is reasonable to expect a large degree of compositional variation and stratification to develop after the introduction of primitive tholeiitic basalt into a subvolcanic chamber. Fractionation processes, possibly dominated by congealation crystallization on the walls and roof, will cause relatively evolved (ferrodioritic) compositions to develop due to disparate rates of thermal and chemical diffusion. As a

result of such processes, trends similar to the one indicated on Fig. 1 for the basic components will be produced.

The genesis of the acid rocks which are found in close association with the basic rocks of the BTVP has been the subject of much discussion; it appears that both fractional crystallization of tholeiitic basalt and partial melting of sialic country-rock have contributed to their genesis^{1–4,24,36,37}.

With these various aspects having been established and in the light of the new whole-rock compositional data presented above, a model is proposed which first considers a metastable situation whereby acid magma (2.3 g cm^{-3}) is ponded above a strongly fractionated, stratified tholeiitic magma, which grades towards ferrodioritic compositions. This situation is represented by Fig. 5*a*. Such subvolcanic chambers are not necessarily closed systems and the incoming of a batch of new primitive tholeiitic basalt magma will have important implications. First, its density will typically be less than that of the ferrodioritic magma which is already present in the chamber, and hence gravitational equilibrium will not ensue (Fig. 5*a*). Such a situation will lead to turbulent mixing processes between the incoming magma and the evolved ferrodioritic liquid (Fig. 5*b*, A). Heat will be transferred between the two, with the net effect of increasing the temperature and decreasing the density of the ferrodioritic magma. The extent to which mixing takes place will depend on both the quantity of new primitive magma introduced, as well as its Reynolds number (which is a function of parameters such as flow velocity and viscosity and density contrast between the two magmas).

As a consequence of these mixing processes there will be an overall increase in the temperature of what will then be a convecting, Fe-enriched, basic magma. This liquid will then interact with the lower-temperature acid magma above due to the formation of double-diffusive layers as heat is lost upwards³⁵ (Fig. 5*b*, B). Cooling of the basic liquid against the acid magma will inevitably take place and mixing will occur, although the extent to which such processes take place will be extremely variable. For example, partial mixing may produce associations such as net-veined complexes. Effusion of acid volcanics (rhyolites, ignimbrites and so on) may also occur as a consequence of devolatilization processes involving the low-density acid magma^{14,38}.

Reductions in the volume of magma remaining in the subvolcanic chamber will lead to gravitational disequilibrium. At this stage it is likely that steeply dipping fractures will propagate upwards and that a central block will subside to recreate lithospheric pressure equilibrium (Fig. 5*c*). If such fractures reach the Earth's surface a caldera will develop^{10,38}. During this stage mixing processes will continue because of the large amount of mechanical energy involved as the central block subsides. Contemporaneous with this subsidence will be the development of associations such as the marscoite ring-dyke, Western Red Hills⁷ and the Kilchrist Ring-dyke, Eastern Red Hills¹⁴, both of Skye (Fig. 5*c*). Accompanying the injection of these mixed magmas

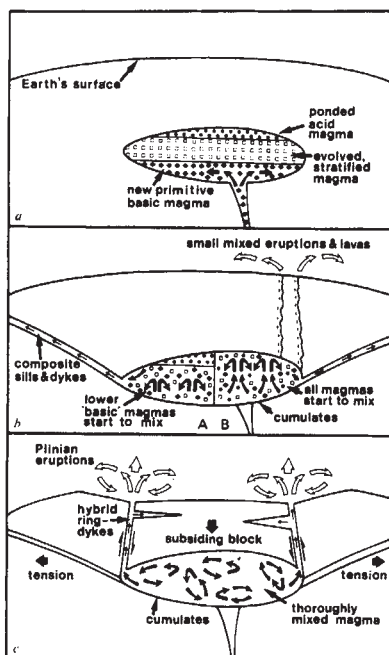


Fig. 5 Postulated events in a hypothetical BTVP subvolcanic magma chamber. *a*, Acid magma ponded above evolved, stratified magma (tending towards ferrodioritic compositions). New primitive basic magma is introduced below. *b*, A, The new buoyant, primitive magma will start to mix with the upper stratified magma. At this stage, magma can be tapped to form composite sills and dykes. *b*, B, The lower convecting layer will eventually start to interact with the ponded acid magma, giving rise to an inhomogeneous hybrid liquid which may be erupted as mixed pyroclastics or lavas. *c*, Eventually, a central block will subside along ring fractures to recreate lithospheric pressure equilibrium, thus causing further extreme magma interaction. This liquid can migrate up to the ring fracture(s) and give rise to Plinian eruptions of mixed magma, as well as ring-dykes (such as the Kilchrist Ring-dyke, Eastern Red Hills, Skye¹⁴).

along ring-dyke fractures there will be eruptions of mixed magma of a more violent nature^{14,21}.

This model is consistent with the field relationships of acid-basic associations, their whole-rock geochemistry and the mechanisms which are considered to be involved in the development of subvolcanic magma chambers. As our knowledge of the last of these three aspects increases, especially with respect to diffusion of various species between contrasting silicate melts³⁹, it will be possible to quantify several of the parameters involved in mixing processes and thereby gain a better under-

standing of the various facets of magma interactions in different tectonic environments. A significant step towards understanding the physical and chemical aspects of these processes is the realization that stratification of the magma system develops and that the basic component evolves towards ferrodioritic compositions before mixing takes place.

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1982 eruption of Mount Cameroon, West Africa

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Mount Cameroon erupted in October 1982 after a 23-yr period of quiescence. The eruption lasted for 24 days and produced 10^7 m^3 of basanite lava from a fissure on the south-west flank. The lavas showed a steady variation in chemical composition with the first lavas slightly more evolved than the last. This chemical variation can be explained by mixing a reservoir magma very close in composition to the 1959 lava with new magma slightly more basic than the last 1982 lava. The size of the pre-inflation magma reservoir may be estimated from mass-balance calculations and must lie in the range 2.3×10^6 – $1.3 \times 10^7 \text{ m}^3$.

MOUNT Cameroon is a large (4,095 m) basanite composite volcano situated on the west African coast (Fig. 1) and is the only active member of the volcanic Cameroon line^{1,2}. The earliest well-authenticated eruption occurred in April 1909 on the northern flank^{3,4} although earlier eruptions are reported to have been seen by the local population and from ships at sea^{5–7}. The first recorded ascent of the mountain was by a party led by Richard Burton⁸ in 1862.

Four further eruptions have occurred this century. A small summit eruption in February 1922 was followed by a much larger emission of lava from craters on the western flank⁹. In June 1954 a summit eruption, lasting about 3 weeks, produced ash and bombs but no lava flow¹⁰. A large lava flow was, however, produced 5 yr later, in February 1959, from a crater on the northeastern flank^{11–13}. The latest eruption started on 16 October 1982 on the south-west flank and lasted until 9 November.

1982 eruption

Lava and ash were erupted from a SW-NE-trending fissure at a height of ~2,700 m on the south-west flank of the volcano. The fissure extended down slope for a distance of ~1 km.

Strombolian activity at its upper end (2,770 m) rapidly built a cinder cone which, by 7 November, had reached a height of 25 m with a rim diameter of 80 m. During the first few days of the eruption¹⁴, the vent emitted fire-fountains up to 300–400 m high. The new cone is situated at 4.163° N, 9.128° E at a point 1.5 km WSW of the prominent (3,094 m) peak referred to by Burton⁸ as Mount Isabel. The lava entered the rain forest at 2,400 m, 1.5 km below the cone, and covered a total distance of ~8 km (Fig. 1). A typical aa flow-field with an aspect ratio¹⁵ of 130 was produced. The distal front of the lava, at ~1,000 m above sea level, measures ~200 m across and 20 m high¹⁴.

At some time between 30 October and 4 November a landslide occurred, unobserved, below the cone producing an elongate depression trending down slope and measuring ~500 m long, 200 m wide and up to 150 m deep. The landslide produced a debris flow composed of old volcanic material (fine ash and blocks of lava) mixed with the disrupted remains of 1982 lava flows. The debris flow covered the upper part of the 1982 lava and came to a halt just above the tree line, 1.5 km from the cone. The flow terminated with a sharply-defined lobate front and, when observed on 9 November, was dry and powdery. The debris clearly did not flow as a slurry with water although heavy

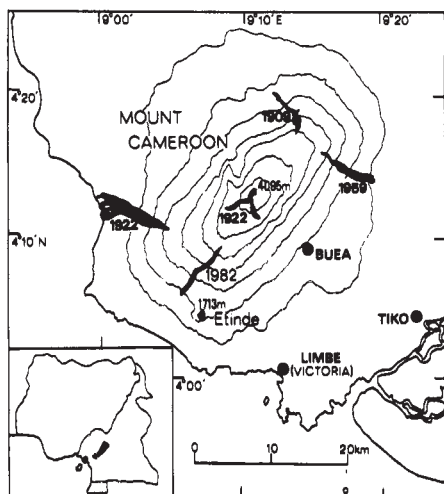


Fig. 1 Map of Mt Cameroon showing the lava flows (black) produced by recorded eruptions.

rain was reported on 31 October. The volume of debris is difficult to estimate because of lack of accurate information on the pre-landslide topography but must be between 2 and $5 \times 10^6 \text{ m}^3$.

After the landslide, numerous small overlapping lava streams, from ~ 5 to 50 m wide, 1 to 8 m high and $<1.5 \text{ km}$ long, flowed into the depression from a small vent outside the base of the disrupted cone. The tongues flowed over and around the debris pile and, in two places, formed cascades, 20 and 40 m high, over the steeper ground.

The sequence of events in the eruption is shown in Fig. 2. These sketch maps are based on our own observations (7–10 November) and on photographs taken by E. Mbwaye, the government photographer in Buea (27 October) and D. Kergomard of Elf Serepca, Douala (30 October).

Physical characteristics

The lava flow observed during the first day of activity¹⁴ formed a stream 3 m wide moving at 20 km h^{-1} . This implies an initial rate of magma discharge of between 10 and $30 \text{ m}^3 \text{ s}^{-1}$. For the last 24 h of the eruption the corresponding value was $\sim 0.1 \text{ m}^3 \text{ s}^{-1}$. During the early stages of cone formation (16–19 October) the implied¹⁴ mean rate of tephra production was $\sim 0.1 \text{ m}^3 \text{ s}^{-1}$ (200 kg s^{-1}). Assuming a maximum rate of between 0.1 and $1 \text{ m}^3 \text{ s}^{-1}$ to have been associated with the phase of fire-fountaining to heights of 400 m , the theoretical modelling of Wilson and Head¹⁶ suggests a maximum initial water content for the magma of $0.6 \text{ wt}\%$.

The temperature of the lava was measured with a Cr–Al thermocouple on 8 November, $\sim 3 \text{ m}$ behind the active flow front which was 3.5 m wide, 1.25 m thick and advancing steadily with a mean velocity of 10^{-2} m s^{-1} down an 18° incline. At this point, $\sim 900 \text{ m}$ from its source, the temperature of the lava increased from $1,045 \pm 5^\circ \text{C}$ to $1,070 \pm 5^\circ \text{C}$ between 15 and 40 cm into its lateral margin. Even if the lava had been erupted at its estimated liquidus temperature ($1,160^\circ \text{C}$), therefore, the mean drop in temperature of the flow interior cannot have exceeded 0.1°C per metre travelled, suggesting an average cooling rate in the range 10^{-1} – $10^{-3}^\circ \text{C s}^{-1}$.

The bulk rheological properties of the lava in the vicinity of its snout (where the ambient shear rates of the deforming layers are estimated to have been between 10^{-1} and 10^{-2} s^{-1}) may be approximated to those of a Bingham material. Using a form of the Jeffreys formula¹⁷, modified to the case of a simple Bingham plastic (C.R.J.K., unpublished calculations), the bulk yield strength and Bingham viscosity of the lava are calculated to have been in the order of 10^4 N m^{-2} and 10^4 N s m^{-2} respectively.

The total volume of lava erupted, estimated from the final dimensions of the lava flows, is $\sim 10^7 \text{ m}^3$. The volume of the new cone is estimated to be $\sim 4 \times 10^5 \text{ m}^3$, which, allowing an additional 50% to include the surrounding ejecta blanket and lost fine material¹⁸, suggests a value of $\sim 4\%$ for the explosivity index^{19,20} of the eruption.

In conjunction with the data on Mt Cameroon's four previous recorded eruptions (refs 3–6, 9, 10 and J. E. Guest, unpublished field data), the timing and volume of the 1982 event are consistent with the volcano having achieved an equilibrium or steady state, with a mean rate of discharge of $0.02 \pm 0.01 \text{ m}^3 \text{ s}^{-1}$. Assuming that this behaviour will continue in the future, extrapolation of a plot of the cumulative volume of magma erupted with respect to time (since 1909), a technique applied to several other volcanoes^{21–23}, suggests that the next high-risk period for a major eruption (of 10^7 m^3 or more of lava) will be between AD 2003 and 2010. This, however, does not preclude the possibility of an earlier, and probably smaller, or later, and probably larger eruption; nor can we identify the likely location of activity.

Composition of magma

Eight rock samples were collected for analysis covering, as far as possible, the full time span of the eruption. These were supplemented by a sample of lava (C212) collected at an early stage in the eruption by F. Le Guern. Our samples are believed to be representative of the eruption.

The debris flow formed a useful marker horizon for distinguishing early lava flows from those erupted later. Two pre-landslide samples (in addition to C212) were collected. C199

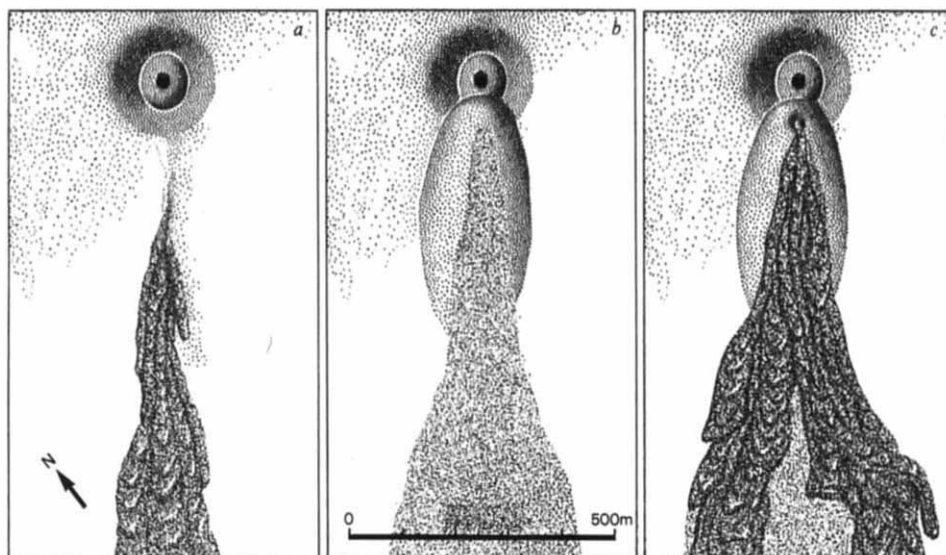


Fig. 2 Sketch maps showing the progress of the 1982 eruption. a, Situation on 27 October. b, The debris flow produced by a landslide at some time between 30 October and 4 November. c, Situation at the end of the eruption on 9 November.

Table 1 Chemical analyses of samples from the 1922, 1959 and 1982 eruptions of Mt Cameroon

	1959		1922			1982							Model
Wt%	C1	C25	C26	C192	C193	C194	C195	C196	C197	C199	C200	C212	C212*
SiO ₂	46.21	43.47	43.27	44.70	44.80	44.81	44.87	44.65	44.72	44.87	44.61	45.12	45.24
Al ₂ O ₃	16.04	12.05	13.14	15.07	15.10	15.13	15.14	15.23	15.45	15.42	14.99	15.44	15.44
Fe ₂ O ₃	4.13	3.47	3.57	3.84	4.81	3.32	3.81	3.59	2.21	3.17	4.53	3.48	12.05†
FeO	6.80	9.11	9.22	8.70	7.82	9.14	8.64	8.85	10.12	9.17	8.02	8.92	
MgO	5.34	10.64	8.95	6.02	6.11	6.01	6.21	6.01	5.91	5.84	6.16	5.94	5.96
CaO	10.12	11.64	11.70	11.86	11.96	11.92	11.93	11.65	11.48	11.48	11.87	11.45	11.33
Na ₂ O	4.59	3.19	3.40	3.68	3.66	3.62	3.54	3.69	3.94	4.02	3.59	3.85	3.87
K ₂ O	1.77	1.40	1.54	1.31	1.27	1.28	1.27	1.35	1.40	1.41	1.30	1.43	1.43
TiO ₂	3.15	3.08	3.39	3.39	3.40	3.40	3.37	3.38	3.44	3.40	3.39	3.40	3.42
MnO	0.21	0.21	0.21	0.20	0.20	0.20	0.19	0.20	0.20	0.20	0.19	0.20	0.20
P ₂ O ₅	0.81	0.73	0.77	0.56	0.54	0.55	0.55	0.59	0.61	0.62	0.55	0.63	0.63
Total	99.17	98.99	99.15	99.34	99.67	99.37	99.52	99.20	99.48	99.60	99.20	99.85	
p.p.m.													
Ni	45	181	137	54	52	53	54	56	49	52	59	58	53
Cr	60	478	330	55	56	56	57	50	39	43	64	48	69
V	216	286	314	347	345	337	335	361	336	332	345	343	314
Sc	15	33	30	27	29	27	27	27	23	23	27	25	23
Cu	67	61	71	101	99	101	101	98	100	98	98	95	90
Zn	100	112	112	104	102	111	108	114	117	111	105	115	110
Sr	1,130	990	1072	871	837	886	885	926	974	976	903	985	962
Rb	45	36	38	29	28	29	29	32	33	34	32	33	34
Zr	409	361	376	304	298	308	307	323	335	340	312	343	339
Nb	102	93	98	73	70	72	72	77	81	82	74	83	81
Ba	599	479	512	410	414	397	414	418	443	456	404	453	473
La	87	83	87	61	61	60	59	66	66	70	59	68	68
Ce	184	167	180	124	120	129	129	135	143	140	131	147	146
Nd	76	69	74	54	51	57	55	59	61	59	55	62	62
Y	35	30	30	29	28	29	29	30	30	32	30	31	31
U	2.38				1.56	1.54	1.56		1.77	1.78	1.63	1.83	1.82
Pb	4.10						2.98		3.46		2.89	2.70	3.32

Precision of analyses (2 σ). Major elements better than 1% except for Na₂O (3%) and MnO (5%).

Sr, Zr, Nb, Pb and U, 1% or less. Cr, Ni, Cu, Zn, Ba, Ce, Nd, 1–2%. V, Sc, Rb, La, Y, 2–3%.

C1, 1959 lava from flow front at Ekona.

C25, C26, 1922 lava from flow front near coast near Bibundi.

C192–C212, from 1982 eruption. Localities and eruption dates given in text.

* 67.5% C195 + 31.8% C1 + 0.65% magnetite from C1.

† Total Fe as FeO.

was collected from a block in the debris flow and so its eruption date cannot be fixed very precisely. C197 was taken from a lava flow partly covered by the debris deposits and recognizable on photographs taken on 27 October.

Of the post-landslide samples, three were seen to erupt (C193 on 7 November and C194 and C195 on 8 November). The last two samples were collected while the flow was still molten. C200 was collected on 9 November from a large lava flow which was still too hot to walk on. C192 is part of a 50-cm bomb, collected 100 m east of the new cone, which had burnt the grass around itself. The burnt grass still retained the form of the blades and so the eruption of this bomb must have post-dated the last heavy rain (31 October). The remaining sample (C196) is made up of small (1 cm) tephra fragments collected 80 m east of the cone and whose eruption date is relatively unconstrained.

The samples are virtually identical petrographically, differing only in the degree of crystallinity of the groundmass. The samples have very sparse (<1%) phenocrysts of olivine (Fo₈₃) and titaniferous augite (TiO₂ 1.3–4.4 wt%, Al₂O₃ 4.2–8.6 wt %) both up to 4 mm in size and small (up to 0.4 mm) phenocrysts of titanomagnetite. The groundmasses are often glassy with microlites and microphenocrysts of olivine (Fo_{78–79}), augite (similar in composition to the phenocrysts), plagioclase (An_{65–70}) and titanomagnetite. The olivine microphenocrysts are usually skeletal or rod-like implying rapid growth in the conduit or lava flows. In contrast, the rare olivine phenocrysts are rounded and often embayed. They are very uniform in forsterite content except for the outer 40 μ m where they zone rapidly to the groundmass olivine composition. The augite

phenocrysts and microphenocrysts show strong oscillatory zoning.

The nine samples from the 1982 eruption were analysed along with two samples of lava from the 1922 eruption (C25 and C26) and one sample from the 1959 eruption (C1). Major and trace elements (except Pb and U) were determined at Edinburgh using X-ray fluorescence techniques (FeO was determined wet-chemically); Pb and U (by isotope dilution) and isotope ratios were determined at Leeds. The analyses are presented in Tables 1 and 2.

The 1982 magma was of basanite composition with ~11% by weight of nepheline in the norm. The analyses show a small but significant change in composition during the course of the eruption with early, more evolved lavas (C212), changing progressively to more basic lavas (C195). The variation was most pronounced for the incompatible elements, with concentrations falling by 6% (Y) to 15% (U) of the amount present in the early lava (C212). This variation is illustrated in Fig. 3 using Nb and K₂O as examples, although a similar pattern is shown for all the incompatible elements. Variation among the more compatible elements is much less pronounced. Some (such as MgO and CaO) show systematic changes with time but others (such as TiO₂ and total Fe) remain constant (Table 1).

Crystal fractionation

Because the lavas are almost aphyric, differences in phenocryst content cannot explain this chemical variation. Attempts to model the variation by simple crystal fractionation processes

were unsuccessful. Least-squares mixing calculations²⁴ using only the observed phenocryst phases (olivine, titanite and magnetite) gave very poor solutions. To produce the variation in incompatible elements with only very slight changes in the more compatible elements, plagioclase had to be included as a fractionating phase. The best solution obtained (sum of squares of residuals = 0.04) required the extraction 6.1% augite, 4.1% plagioclase (An_{65}) and 1.4% magnetite from C195 to produce C212. This model, however, is unreasonable because plagioclase is not observed as a phenocryst phase. Furthermore, Sr behaved incompatibly in the 1982 magma. Its concentration fell by 10.2% during the eruption compared with 10.5% for Zr. The extraction of calcic plagioclase from the magma in the amount predicted by the crystal fractionation model would have caused Sr to behave compatibly. It is possible that crystallization of a high pressure, aluminous pyroxene could account for the magma variation but it is difficult to envisage liquids rising from such a deep magma chamber without being mixed on the way to the surface. The compositional zoning in the magma chamber implied by the 1982 lavas is therefore unlikely to be the result of crystal fractionation.

Magma mixing

Magma mixing offers a much more plausible explanation. The variation can be modelled very precisely by mixing the last and most basic of the 1982 lavas (C195) with the 1959 lava sample (C1). This is illustrated in Fig. 4 where the linear array of 1982 compositions lies very close to a mixing line between C195 and C1. It is significant that the two samples of 1922 lava (C25, C26) lie well away from this mixing line.

Most of the elements analysed plot along similar mixing lines, the only exceptions being Fe, Ti, V and Zn. The concentrations of these four elements in the 1959 sample C1 are slightly lower than predicted by the mixing model. These elements, however, are all concentrated in magnetite which is a liquidus phase in both the 1959 and 1982 magmas. Sample C1 contains magnetite phenocrysts (up to 0.5 mm), often included within olivine and augite phenocrysts. It is quite possible, therefore, that the magma left behind after the 1959 eruption was slightly cumulus-enriched in magnetite compared with the sample C1. The mean of four microprobe analyses of magnetite in C1 gave total Fe as FeO, 66.4%; TiO₂, 18.5%; Al₂O₃, 5.8%; MgO, 4.9%; MnO, 0.62%; V, 2,750 p.p.m.; Zn, 690 p.p.m.; Cr, 1,680 p.p.m.; and Ni, 320 p.p.m. A 2% enrichment of C1 in magnetite of this composition would remove the discrepancy in these elements.

Least-squares mixing calculations²⁴ show that the major-element composition of the early lava sample (C212) can be modelled very closely (sum of squares of residuals = 0.03) by mixing 67.5% C195, 31.8% C1 and 0.65% magnetite. The results of the mixing calculation are shown in Table 1 alongside the analysis of C212. The agreement is excellent and within the analytical precision for most elements. This close agreement, for trace elements (which were not included in the least-squares calculations) as well as for major elements, provides strong evidence in favour of a magma mixing model and suggests that

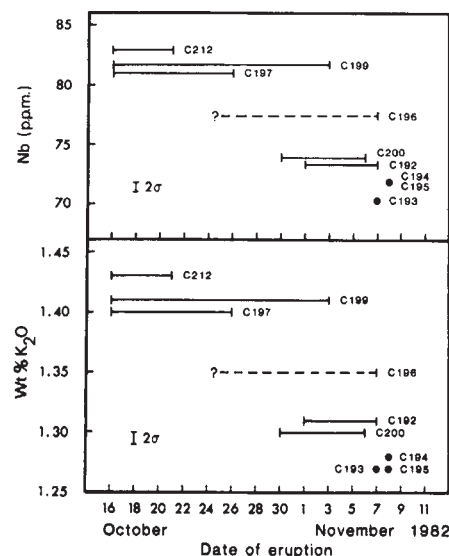


Fig. 3 Variation in Nb and K₂O content of the magma during the course of the eruption. Similar variation is shown by the other incompatible elements (Table 1). Horizontal bars denote uncertainty in eruption date.

C1 is close to the composition of the reservoir magma. It is, however, possible that the lavas erupted in 1959 showed chemical variation and that C1 is not representative of the reservoir magma. In this case, the 1959 lavas would have to plot along mixing lines (or their extrapolations) between C195 and C1 for all elements. If the degree of variation was similar to the 1982 variation (~10% for incompatible elements) then the proportion of reservoir magma in C212 could range from 23 to 59%. This would not invalidate the mixing model. The Mt Cameroon lavas show a wide scatter on variation diagrams (J.G.F., unpublished data) so the probability of some other magma entirely unrelated to the 1959 eruption having a composition capable of producing the agreement shown by the 'model C212' in Table 1 is very low.

Isotopic data

To test the magma mixing model, some of the samples were analysed for Sr and Pb isotopes (Table 2). Isotopic variation in the products of the eruption could only be explained by mixing.

The Sr data are consistent with magma mixing (Fig. 5) although variation within the data from the 1982 and 1959 eruptions is just within experimental error and cannot, therefore, be used to prove mixing. The sample of 1922 lava analysed (C25) is, however, isotopically distinct from most of the 1982 samples suggesting that magma is supplied to the volcano in discrete batches from a slightly heterogeneous source rather than from a single large evolving magma chamber. This is supported by the large differences in incompatible element ratios

Table 2 Isotopic data

	⁸⁷ Sr/ ⁸⁶ Sr	¹⁴³ Nd/ ¹⁴⁴ Nd	²⁰⁶ Pb/ ²⁰⁴ Pb (±0.014)	²⁰⁷ Pb/ ²⁰⁴ Pb (±0.016)	²⁰⁸ Pb/ ²⁰⁴ Pb (±0.06)	²⁰⁷ Pb/ ²⁰⁶ Pb (±0.00027)
C1	0.703342 ± 23		20.363	15.654	40.33	0.76830
C25	0.703362 ± 21					
C195	0.703293 ± 25	0.512792 ± 8	20.284	15.646	40.07	0.77089
C197	0.703301 ± 25		20.315	15.636	40.07	0.76926
C199			20.287	15.636	40.07	0.77071
C200	0.703309 ± 29		20.309	15.643	40.09	0.76982
C212	0.703325 ± 27		20.232	15.659	40.06	0.77353

Errors on Sr and Nd isotope ratios are 2σ within-run errors; Pb isotope errors are estimated from reproducibility of SRM981. Pb isotope ratios are normalized to SRM981 (about 0.06% AMU), ¹⁴³Nd/¹⁴⁴Nd to ¹⁴⁶Nd/¹⁴⁴Nd = 0.7219 and ⁸⁷Sr/⁸⁶Sr to ⁸⁶Sr/⁸⁸Sr = 0.1194. SRM987 ⁸⁷Sr/⁸⁶Sr = 0.710258 ± 6; BCR-1 ¹⁴³Nd/¹⁴⁴Nd = 0.512657 ± 9 (2σ).

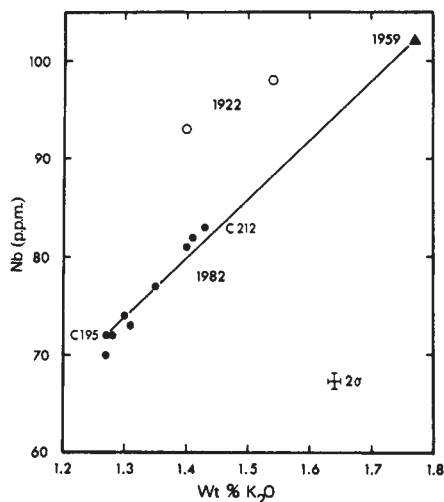


Fig. 4 Chemical variation in the 1982 samples (●) compared with a mixing line between the last lava erupted (C195) and a sample of 1959 lava (C1, ▲). C212 is the earliest of the samples. Two analyses of 1922 lavas (○) are included for comparison.

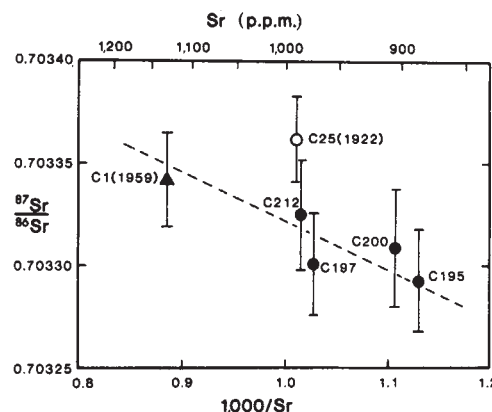


Fig. 5 $^{87}\text{Sr}/^{86}\text{Sr}$ variation among the Mt Cameroon lavas. The broken line is the best fit to the 1982 and 1959 data. Mixing would produce linear relationships on this diagram. Bars represent 2σ error; errors in the Sr determination lie within the size of the data points.

between the 1922 lavas and the lavas of the 1959 and 1982 eruptions (Fig. 4).

Of the Pb isotope data, only $^{207}\text{Pb}/^{206}\text{Pb}$ varies outside the precision of measurement. This is the most precisely determined ratio because of the small mass difference in the two isotopes. The small variation in this ratio, however, shows no correlation with order of eruption. Pb also appears to vary randomly in the magma (Table 1) unlike the other incompatible elements. We can offer no explanation for this slightly erratic behaviour of Pb and its isotopes. The data, however, do not contradict the magma mixing model.

The combination of relatively unradiogenic Sr ($^{87}\text{Sr}/^{86}\text{Sr} = 0.7033$) with highly radiogenic Pb ($^{206}\text{Pb}/^{204}\text{Pb} = 20.3$) is rather unusual, and so, for completeness, $^{143}\text{Nd}/^{144}\text{Nd}$ was determined on the most basic 1982 sample (C195). The ratio corresponds to ϵ_{Nd} of +3.0, appreciably lower than that ($\sim +6$) of most modern oceanic basalts with similar $^{87}\text{Sr}/^{86}\text{Sr}$. These data are very similar to those reported from St Helena by White and Hofmann²⁵, where Pb is even more radiogenic²⁶. This suggests that the unusual mantle source required to explain the St Helena data also contributed to magma genesis in the Cameroon line, the southwestward extension of which intersects the mid-Atlantic ridge close to St Helena. The Mt Cameroon Pb isotope data do not, however, fall on a mixing trend between St Helena and mid-ocean ridge basalt Pb on either the $^{207}\text{Pb}/^{204}\text{Pb}$ or $^{208}\text{Pb}/^{204}\text{Pb}$ versus $^{206}\text{Pb}/^{204}\text{Pb}$ diagrams, but instead lie close to the Pb compositions of basalts from Guadaloupe, an island in the Pacific Ocean²⁶.

Compositional zonation

The compositional variation in the products of the 1982 eruption suggests a vertical zonation in the composition of the magma in the reservoir. Mixing calculations suggest that this zonation resulted from the mixing of magma left behind in the reservoir after the 1959 eruption with new and more basic magma.

Examples of progressive variation in magma chemistry during eruption are common among silicic volcanoes²⁷ but are seldom reported from more basic volcanoes. Chemical variation in the course of a single eruption has been reported from Kilauea²⁸, Etna²⁹ and Helgafell on Heimaey³⁰. However, the frequent occurrence, in a wide range of geological environments, of composite dykes and sills, usually with basic cores and more evolved margins (see for example, ref. 31) suggests that compositional zonation in basic magma bodies may be a common phenomenon. In the case of the 1982 eruption of Mt Cameroon

and the 1973 eruption of Helgafell the magma body must have been zoned upwards to more evolved compositions.

Magma densities were estimated from the major element composition of the 1982 lavas using the method of Bottinga *et al.*³². A calculated liquidus temperature³³ of 1,160 °C was assumed and $\text{Fe}_2\text{O}_3/\text{FeO}$ ratios were normalized to 0.22, the value of the most reduced sample (C197). These estimates suggest that the magma reservoir was density-stratified with the earliest magma erupted (C212) 0.005 g cm⁻³ less dense than the last magma (C195). However, this density contrast could be reduced to zero if the last magma erupted was 20 °C hotter than that erupted first.

If the new magma was denser than the magma into which it was injected it would probably form a layer at the bottom of the reservoir and not mix. If, however, the new magma was superheated (20–30 °C above its liquidus) it could have been less dense than the reservoir magma. In this case the two magmas could mix by turbulent flow as suggested by Sparks *et al.*³⁴. This mixing would not be complete because the mixing would cease once the density was equalized throughout the reservoir. The reservoir would, therefore, be hotter at the bottom than at the top and have a compositional gradient which would balance the temperature effect on the density. As heat was lost from the system the temperature gradient would be reduced leaving the hybrid liquid denser at the bottom than at the top. The injection of superheated new magma would account for the sparsity and the rounded and embayed appearance of the phenocrysts. The 1959 lava (inferred to be representative of the reservoir magma) is more porphyritic with $\sim 10\%$ by volume of olivine and augite phenocrysts.

Magma composition and reservoir size

The 1982 lavas were still changing in composition during the last few days of the eruption (Fig. 3) so it is unlikely that any new magma not mixed with reservoir magma was erupted. The new magma must, therefore, have been more basic than C195. If it can be assumed that the reservoir magma had the composition of C1 + 2% magnetite and that the compositional zoning in the reservoir was uniform, then estimates of the composition of the new magma and the size of the magma reservoir can be made. These two quantities are clearly interrelated in these estimates because the more basic the new magma the greater will be the volume of reservoir magma needed to mix with it to produce the observed magma.

The smallest reasonable magma reservoir (and hence the least basic new magma) can be modelled by assuming that 10^7 m^3 of new magma was injected into a small reservoir containing 1959 magma and that by the end of the eruption the reservoir had returned to its pre-inflation size. Mass-balance calculations based on this model require a pre-inflation volume of $2.3 \times 10^6 \text{ m}^3$. The new magma would be only slightly more basic than C195 with 6.30% MgO and 1.23% K_2O .

The largest possible size for the magma reservoir can be estimated by letting the new magma become so basic that the concentration of one of the incompatible elements reduces to zero. This will happen first to uranium with MgO at 7.95% and K_2O 0.29%. $4.4 \times 10^7 \text{ m}^3$ of new magma of this composition would need to be mixed with $2.8 \times 10^7 \text{ m}^3$ of reservoir magma. This composition is, however, unrealistic for alkali basalt magmas. From the composition of analysed samples of Mt Cameroon lavas (J.G.F., unpublished data) it is most unlikely that the new magma (with <8% MgO) would have <0.8% K_2O . $2.6 \times 10^7 \text{ m}^3$ of new magma with 7.07% MgO and 0.8% K_2O would need to be injected into a reservoir with a volume of $1.3 \times 10^7 \text{ m}^3$ to produce the observed magma variation.

Thus the pre-inflation volume is likely to have been in the range 2.3×10^6 – $1.3 \times 10^7 \text{ m}^3$ with the lower volume more likely than the upper volume. These volumes are similar to those estimated by Ryan *et al.*³⁵ for Kilauea. Uncertainties in the

volume of magma erupted and in the assumptions made (the compositional gradient in the reservoir might not have been uniform, for example) are unlikely to change these estimates by more than a factor of 2. Even if C1 is not representative of the reservoir magma as discussed earlier, allowing its composition to vary over a reasonable range ($\pm 10\%$ of its incompatible element concentrations) will not alter these volumes substantially. Taking the least evolved composition, for example, gives pre-inflation volumes ranging from 5.4×10^6 to $1.6 \times 10^7 \text{ m}^3$. It must be concluded, therefore, that the magma reservoir was very small (equivalent to a sphere with a radius in the order of 100 m) and that the new magma supplied to the reservoir was not very basic (MgO <7%). The persistence of magma in such a small chamber for 23 yr requires that the wall rocks be hot. A deep reservoir is therefore more likely than a shallow one and is consistent with the 18 km distance between the sites of the 1959 and 1982 eruptions (Fig. 1).

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Expression of a microinjected immunoglobulin gene in the spleen of transgenic mice

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Transgenic mice were produced by microinjection of a rearranged, functional immunoglobulin κ gene into fertilized mouse eggs and implantation of the microinjected embryos into foster mothers. Mice that integrated the injected gene were mated and the DNA, RNA and serum κ chains of their offspring were analysed. The data from offspring of three different transgenic mice indicate that the microinjected gene is expressed in the spleen, but not the liver of mice which inherited the injected gene.

IMMUNOGLOBULIN variable (V) and constant (C) region genes must be rearranged into close proximity before expression into a functional immunoglobulin protein can occur^{1,2}. The functional rearrangement process takes place only in the B-lymphocyte lineage. To investigate the control of immunoglobulin gene expression we have produced transgenic mice by microinjection³ of an immunoglobulin gene into fertilized mouse eggs. Microinjection of a gene into an embryo introduces that gene into every cell of the resultant mouse and often leads to efficient expression of the injected gene^{4–6}. The presence of the

injected gene in germ cells allows offspring of transgenic mice to inherit the injected gene. Compared with cell transfection experiments, this approach has the advantages of avoiding potential problems due to the transformed phenotype of cultured cells and allowing direct comparison of expression in different tissues that have the injected gene at the same location in the genome.

In our experiments the injected gene is the functional κ gene of the myeloma MOPC-21 (ref. 7). Its variable region, $V_{\kappa}M.21$, is rearranged next to the joining gene segment $J_{\kappa}2$ (ref. 8) and

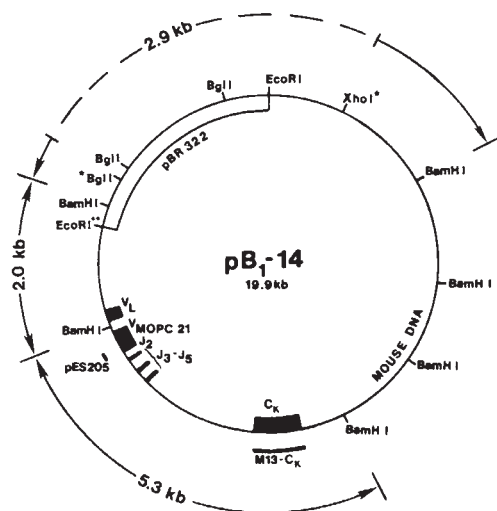


Fig. 1 Map of plasmid DNA pB1-14. The functional MOPC-21 κ gene was initially cloned from genomic DNA into Charon 4A (M21B1)⁷. The $V_{H,M.21}-C_{\kappa}$ gene insert was excised at the *EcoRI* sites and cloned into the *EcoRI* site of pBR322. Because injection of linear DNA with heterologous ends seems to increase the likelihood of integration (R. L. B. and R. Palmiter, unpublished), such a fragment was restricted from plasmid pB1-14 using *BglII* and *XhoI*. The large *BglII**-*XhoI** fragment (14.8 kb) was separated from the smaller *BglII*-*BglII* and *BglII*-*XhoI* fragments by sucrose gradient centrifugation, ethanol precipitated, redissolved in 10 mM Tris pH 7.5, 0.25 mM EDTA and injected. V_L , leader sequence; VMOPC-21, variable region; $J2$ to $J5$, joining segments; C_{κ} , constant region. Probes for $V_{H,M.21}$ (pES205) and C_{κ} (M13- C_{κ}) are indicated. *EcoRI***, the unique *EcoRI* site present in the microinjected pB1-14 gene. The 5.3 kb, 2.0 kb and 2.9 kb *Bam*HI restriction fragments obtained by hybridization with pBR322 and C_{κ} (see Fig. 2b) are indicated; the broken line denotes the portion (5.9 kb) which was eliminated from the plasmid DNA before microinjection. The fact that a 2.9-kb long pBR322 positive *Bam*HI restriction fragment was obtained in DNA of transgenic mice (Fig. 2b) suggests that the free ends of the linear plasmid DNA are joined in the integrated, amplified pB1-14 DNA.

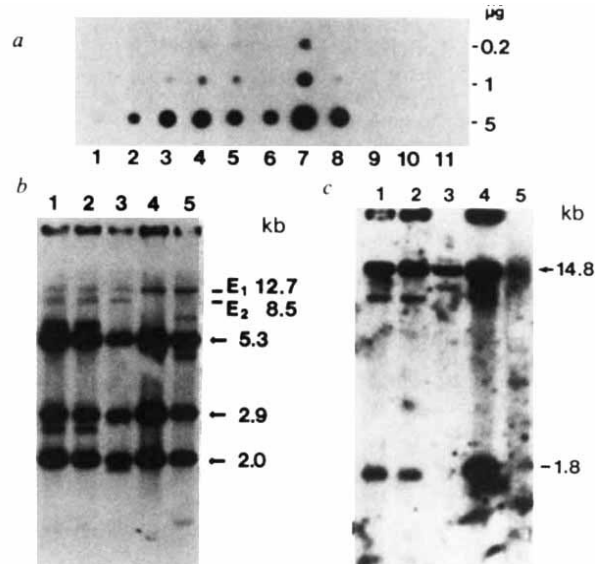


Fig. 2 DNA analysis of transgenic mice (same mice as in RNA and protein analyses in the following figures). *a*, Quantitation of the microinjected gene. The dot hybridizations were probed with pBR322. Each row (top to bottom) contains 0.2, 1.0, 5.0 μ g DNA. 1–3, Normal mouse DNA with 1.5, 7.3 or 29.3 copies, respectively, of 1,000 base pairs (bp) of pBR322 added per cell; 4–8, kidney DNA from transgenic mice: offspring of father A (4, 5), B (6), C (7, 8); 9–11, kidney DNA from normal littermates A, B, C, respectively. *b*, Southern³¹ blot. Each lane contains 30 μ g of kidney DNA digested with BamHI. The blot was probed with C κ and pBR322. 1, 2, Positive mice of transgenic line A; 3, line B; 4, 5, line C. E₁ and E₂, endogenous κ genes of C57BL/6 and 5JL mice respectively. $\rightarrow$, Bands expected from BamHI digestion of plasmid pB1-14 when tandemly integrated in head to tail fashion (see Fig. 1). When the same blot was probed with pBR322 alone, only the 2.9 and 2.0 kb bands were seen (not shown). *c*, Southern blot. Each lane contains 30 μ g of kidney DNA (except lane 5 which contains 30 μ g of tail DNA), digested with *Eco*RI. The blot was probed with pBR322. 1–5, As in *b*.

is contained in the plasmid pB1-14 (Fig. 1). The V_{κ} M.21 portion of the microinjected gene provides a means to distinguish the expression of the introduced gene from that of the endogenous κ genes, both on the RNA and protein levels. The V_{κ} M.21 gene is a member of the V_{κ} 15 family of V_{κ} genes^{9,10}. This gene family has approximately ten members which are sufficiently homologous to cross-hybridize using a V_{κ} M.21 probe¹¹. V_{κ} genes of this family are probably rearranged and expressed in some B lymphocytes in normal mice, and transcripts from endogenous genes might be expected to confound the detection of transcripts from the injected gene. However, transcription of homologous endogenous V_{κ} 15 genes is sufficiently infrequent to be virtually undetectable, as shown below. RNA encoded by the microinjected gene would therefore be uniquely detected by a V_{κ} M.21 probe if it were expressed in a tissue at a normal rate in most cells or at a high rate in a subpopulation of cells. The MOPC-21 κ protein is also sufficiently distinct in molecular weight¹² and isoelectric point (see below) that it can be distinguished from other κ chains in mouse serum, provided it is produced and secreted in some quantity. Analysis of transgenic mice containing the pB1-14 gene shows that expression of the microinjected κ gene is apparent on both the RNA and protein levels.

Production of transgenic mice

DNA for injection was prepared as described in Fig. 1. Eggs from (C57BL/6×SJL) F_1 mice were fertilized with F_1 sperm. The male pronuclei of 300 fertilized eggs were microinjected with 2 μ l containing about 440 copies of the pB1-14 fragment and 192 eggs were inserted into the reproductive tract of random

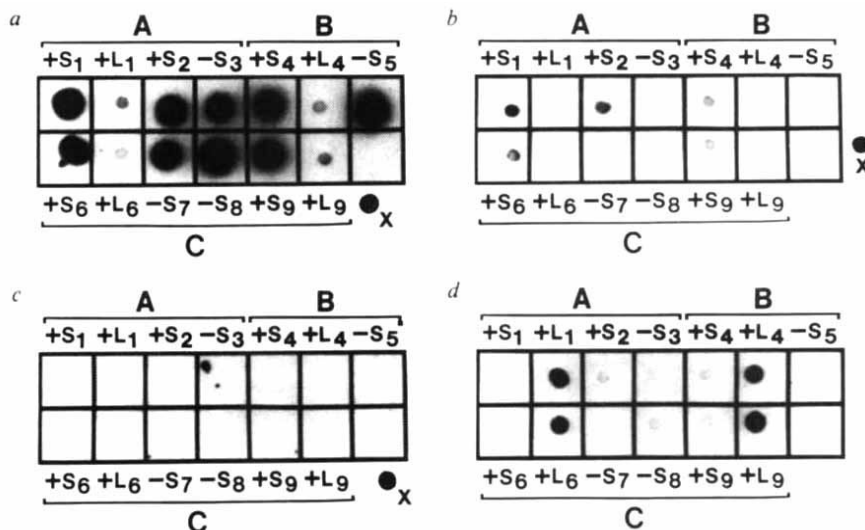
bred Swiss foster mothers⁴. Eleven animals developed from these eggs.

After the mice were weaned, total nucleic acids were extracted from a piece of their tail, and the presence of pBR322 DNA was detected by dot hybridization. Six of the 11 mice had integrated the injected gene. The six transgenic mice, all males, were then mated with C57BL/6 females and the offspring were tested for the presence of the pB1-14 gene. Consistent with mendelian inheritance of a single integration site, approximately 50% of the offspring also contained pB1-14 sequences in amounts relatively equal to their respective fathers. We are reporting here the analysis of first generation offspring from three different transgenic fathers (A, B and C).

Characterization of the microinjected DNA

The integrated genes were characterized with respect to copy number and mode of integration. Figure 2a shows an autoradiogram for an approximate quantitation of the copy number of the integrated gene. Kidney DNA from the offspring of transgenic mice was analysed by dot hybridization with pBR322. The pBR322 sequences were quantitated by cutting the dots out of the nitrocellulose filter and counting them in a liquid scintillation counter. Offspring that inherited the injected gene are shown in lanes 4–8, while littermates that did not inherit the gene are in lanes 9–11. Compared with the DNA standards (lanes 1–3) the two positive offspring of father A contain about 50 copies, the positive offspring of father B contains about 20 copies, while the two offspring of father C differ in their copy number. One C offspring contains about 95

Fig. 3 RNA blots of transgenic mice and normal littermates. Poly(A)⁺ RNA was prepared from spleens (S) and livers (L) and 0.5 µg (a) or 1 µg (b, c, d) after denaturation with glyoxal³² were spotted onto nitrocellulose³³. The spots were hybridized with the following probes: M13 C_κ (contains about 500 bp of C_κ and 3' untranslated region subcloned into M13) (a); pES205 (contains 103 bp of the V_κ region of MOPC-21 subcloned into pBR322) (b); pBR-322 (c); cDNA prepared from total liver poly(A)⁺ RNA (d). Hybridization conditions were 0.45 M NaCl, 0.045 M Na citrate 5 × Denhardt's solution³⁴, 0.1% SDS, 50 µg ml⁻¹ yeast tRNA (Sigma), 2.5 µg ml⁻¹ poly(A), 50 µg ml⁻¹ heat-denatured salmon testis DNA, 25 ng ml⁻¹ ³²P-labelled, nick translated, heat-denatured probe, 10 ml total volume per nitrocellulose filter, 67 °C, 24 h. The specific activity of the probes was about 2 × 10⁸ c.p.m. µg⁻¹. After hybridization the filters were washed exhaustively in 15 mM NaCl, 1.5 mM Na citrate, 0.05% SDS at 65 °C. A, B, C represent three different transgenic mouse lines. Each subscript number represents an individual mouse. +, Transgenic mice, -, negative littermates. X, spot of probe DNA. Exposures to Kodak X-omat film were for 4 days (a), 2 days (b), 5 days (c), 12 h (d).



copies, the other about 40 copies. If in father C the injected gene is integrated in two different locations in the genome, one would expect those two loci to be inherited separately. To distinguish multiple from single integration sites, the kidney DNA of the offspring was analysed by restriction enzyme digestion and Southern blotting. Figure 2b shows a representative autoradiogram. A *Bam*HI restriction site polymorphism between C57BL/6 and SJL causes the endogenous C_κ genes to appear as two bands, E₁ and E₂. The three darkest bands are those expected from a *Bam*HI digestion of concatenated pB1-14 in head to tail integration. Thus in analogy to other transgenic mice⁴⁻⁶ the majority of the multiple copies appear to be integrated in tandem. This is supported by the Southern blot (Fig. 2c) using *Eco*RI which cuts only once in the linearized pB1-14 gene that was microinjected (site *Eco*RI**). An amplified hybridization band of 14.8 kilobases (kb) is seen in the DNA of all five mice. The length of 14.8 kb is that of the linearized pB1-14. The other bands in Fig. 2b and c probably represent junction fragments between pB1-14 and endogenous DNA, as well as some pB1-14 DNA which has suffered a partial deletion (see especially the 1.8 kb band in one offspring (lane 4) of father C). The two mice of line C, besides sharing the major hybridization bands, differ in the respective minor bands produced by *Bam*HI or *Eco*RI digestion. This suggests that there may be at least two different integration sites in the transgenic father C. This seems to be supported by the finding that father C has an approximately additive copy number (not shown). However, details of the integration mode in all the transgenic mice containing the pB1-14 gene need to be further studied.

Expression of microinjected κ gene into RNA

We determined whether the injected κ gene was transcribed by analysing the poly(A)⁺ RNAs of spleen and liver of positive mice and of their normal littermates. Figure 3a shows that the spleens of both positive offspring and their negative littermates contain similar quantities of C_κ containing RNA. However, only the spleens of the positive mice contain detectable RNA having the V_κ region of the injected gene (Fig. 3b). The livers of the positive mice do not contain appreciable amounts of κ RNA (Fig. 3a); only faint hybridization of the C_κ probe with liver RNA was detected after long exposure of the X-ray film. When measured by scintillation counting this represents only 0.6–0.9% of the C_κ RNA found in spleen and could be due to circulating lymphocytes present in the highly vascularized liver tissue (see below). As a control for the quality of the liver RNA, the same RNAs were also hybridized with cDNA prepared from total

liver RNA (Fig. 3d): the prevalent liver specific sequences hybridize strongly with liver RNA and weakly with spleen RNA as expected. There are no transcripts of pBR322 sequences in the poly(A)⁺ RNAs of either spleen or liver (Fig. 3c). Poly(A)⁺ RNAs are also negative for pBR322 (not shown). This result argues against expression via pBR322 sequences.

To evaluate whether the small amounts of κ RNA seen in the livers of transgenic mice could be due to a low level of transcription from the transferred κ gene, comparisons with normal liver RNA were made. It was found that livers from normal mice also contain small amounts of κ RNA (not shown) probably due to circulating lymphocytes. It is therefore apparent that the microinjected κ gene is not transcribed in the liver of transgenic mice at a detectable level.

To determine whether κ RNAs in the spleens of transgenic mice were of correct size, Northern blots were performed with total spleen RNAs (Fig. 4). Both V_κM.21 and C_κ sequences of transgenic spleens were found to be in RNA of mature size, the same as that of κ mRNA of normal spleen (Fig. 4 and ref. 13). In analogy to the results with dot hybridization of poly(A)⁺ RNAs (Fig. 3), the total spleen RNA of normal mice does not show detectable levels of V_κM.21 RNA (even after extensive exposure of the X-ray film, not shown). These data favour accurate initiation and termination of transcription from the microinjected pB1-14 gene.

A comparison was made of the relative expression of the microinjected gene and endogenous κ genes (Fig. 5). The results of approximate quantitations using dot hybridizations are shown in Fig. 5. These data are only approximate because the V_κM.21 probe is only about 100 nucleotides long (one-fifth the length of the C_κ probe). For this reason, a larger quantity of spleen RNA and standard DNA was used for the V_κM.21 hybridization than for the C_κ hybridization. Thus the hybridization conditions are not identical. After exposure of the dot hybridizations to X-ray film, the dots were cut out and counted in a liquid scintillation counter. When RNA quantities were normalized by comparison with the DNA standards, it was found that the V_κM.21-containing RNA represents about 50% of the total κ RNA in the spleens of positive mice, but less than 4% of total κ RNA from normal littermates. In addition we found that spleens of the transgenic mice contain 1.5 to 2 times as much κ RNA in proportion to total poly(A)⁺ RNA as spleens of the normal littermates. Since in the normal spleen approximately 6,000 κ RNA molecules are produced per average cell¹⁴ spleen cells of the transgenic mice may synthesize as many as 3,000 pB1-14 encoded κ RNA molecules per cell. These relatively high levels of expression are similar to the levels of growth

hormone RNA in the most actively transcribing transgenic mice which contain the metallothionein-growth hormone fusion gene⁶.

Serum κ chains encoded by transferred gene

The MOPC-21 κ gene codes for a L chain of relatively high molecular weight¹², and because of the unique V_{κ} M.21 region the L chain has a distinct isoelectric point. The protein is apparently glycosylated and produces two spots of characteristic size, shape and location in two-dimensional (2-D) protein gels¹⁵. Figure 6a shows the 2-D gel pattern of purified MOPC-21 immunoglobulin. The light chain appears as two spots at about 29,000 molecular weight (29K) (just below the 30K marker). Figure 6b-d shows 2-D gels of the sera of transgenic mice and normal littermates. Differences in intensities and location of many of the spots may be due to allelic differences in serum proteins in these backcrossed mice. This makes a one-to-one correlation difficult.

However, in keeping with their apparent monoclonal origin, the characteristic two MOPC-21 protein-like spots seem to be relatively invariable, where they occur. Figure 6b shows the characteristic spots in the serum from positive offspring number 1 of transgenic mouse A. Figure 6c shows the absence of such spots in a littermate negative for pB1-14 DNA. We have tested the sera of a total of 13 transgenic mice and of 11 normal littermates (some additional examples are shown in Fig. 6d). Although normal mice probably express V_{κ} M.21 genes at a low rate, we have not seen the two characteristic spots in our conditions in normal sera, but consistently find them in the sera of transgenic mice. Because these gels are run in reducing conditions, it is not yet determined whether the injected light-chain gene is expressed in the serum in association with heavy chain, or as free light chain, or both.

Discussion

These results from mice that are transgenic for a functionally rearranged κ gene have several implications concerning control of expression of the injected gene with respect to amount, tissue specificity, B-cell ontogeny and post-transcriptional events. In contrast to immunoglobulin genes used in transfection experiments, our injected gene contains no attached viral sequences which could potentially enhance transcription. Furthermore, pBR322 sequences appear not to be involved in transcription of the injected κ gene. Also, since the injected gene is equivalently expressed in different transgenic mice the exact mode of integration and copy number appear to have little bearing on expression. So far, the behaviour of the injected gene seems to reflect the function of a normal functionally rearranged immunoglobulin light-chain gene.

Expression of the injected κ gene at a relatively high rate in all transgenic mice so far examined was an unexpected finding. In transgenic mice obtained by others with several different genes, expression was low or absent¹⁶⁻¹⁸. Consistently successful expression was only obtained with fusion genes of the metallothionein (MT) promoter and either the herpes simplex thymidine kinase (*tk*) gene or the rat growth hormone gene⁴⁻⁶. However, in the MT-*tk* system the expression was variable in offspring which apparently contained the same number and arrangement of the microinjected genes as their transgenic parents. Perhaps the expression signals of immunoglobulin genes are particularly strong. Examination of more transgenic mice is under way and may reveal an as yet undetected variability of expression.

While only spleen and liver have been studied so far, it appears that the microinjected pB1-14 gene may be expressed in a tissue-specific fashion. We presume that the cells in the spleen which express this gene are B lymphocytes, although this has not yet been shown directly. Since immunoglobulin genes are not rearranged in liver cells, for the first time in these transgenic mice liver cells contain a functionally rearranged and potentially expressible immunoglobulin gene. Hepatocytes, however, do

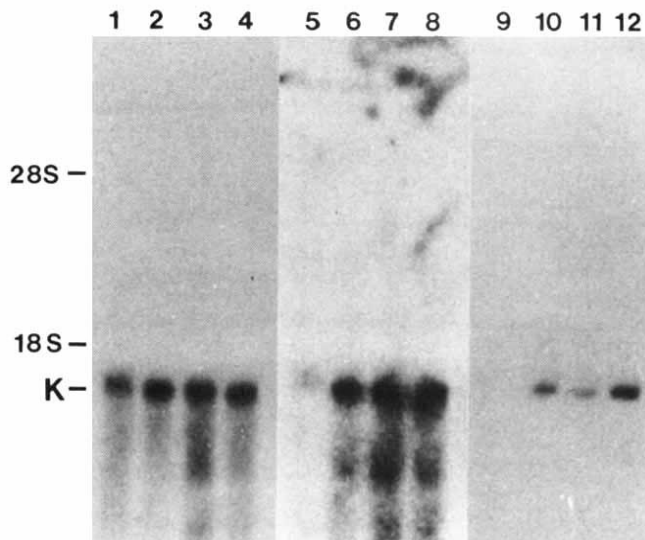


Fig. 4 Northern blot³³ of total glyoxal treated³² spleen RNAs (30 μ g per lane) of a pool of five normal mice (1, 5 and 9) and pools of three, five and three positive offspring from three different transgenic mice respectively (2, 6 and 10; 3, 7, and 11; 4, 8 and 12). Hybridization conditions were as in Fig. 3; probes were M13 C_{κ} (1-4) and pES205 (V_{κ} M.21) (5-12). Sizes of 28S and 18S ribosomal RNAs were determined from total mouse myeloma RNA electrophoresed in the same gel and then stained with ethidium bromide. After hybridization with C_{κ} the blot of 1-4 was treated in 90% formamide at 65 °C for 3 h to remove most of the probe and later rehybridized with V_{κ} M.21 (tracks 5-8). The small amount of radioactivity in lane 5 is from some remaining C_{κ} probe which gave the same signal after reexposure to X-ray film before hybridization with V_{κ} M.21 (not shown). The RNAs of tracks 9-12 were electrophoresed on a different gel and hybridized only with V_{κ} M.21.

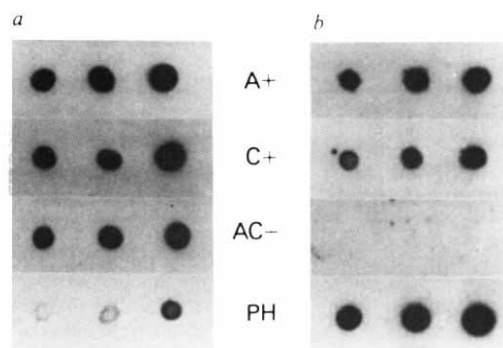


Fig. 5 Relative quantities of C_{κ} and V_{κ} M.21 RNAs in spleens of transgenic mice and normal littermates. *a*, Probed with M13 C_{κ} . The spleen RNAs were 200 ng, 400 ng and 800 ng. *b*, Probed with pES205. The spleen RNAs were 0.5 μ g, 1 μ g and 2 μ g. **Methods:** Spleen poly(A)⁺ RNAs of the two positive offspring A were pooled, as were the poly(A)⁺ RNAs of offspring C. As controls, spleen RNA of negative A and C offspring were pooled together. Dot hybridizations of these RNAs included as a standard the DNA of the M21B₁ clone, and were hybridized with a C_{κ} or V_{κ} M.21 probe. A⁺: pool of spleen poly(A)⁺ RNAs of transgenic mice 1 and 2 (see Fig. 3); C⁺: pooled spleen poly(A)⁺ RNAs of transgenic mice 6 and 9. AC⁻: pooled spleen poly(A)⁺ RNAs of negative littermates 3, 7 and 8. PH, DNA of phage clone M21B₁ (see Fig. 1), 1, 2 and 4 ng (in *a*), 10, 20 and 40 ng (in *b*). Exposure to X-ray film: 14 h.

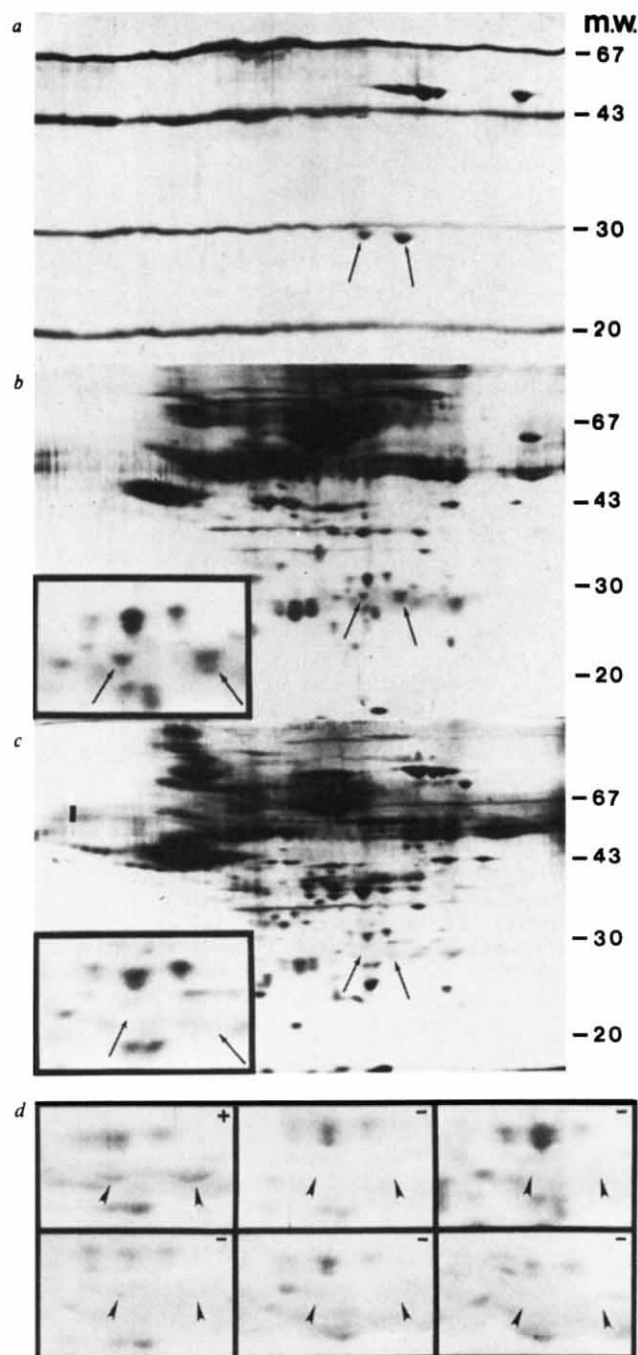


Fig. 6 Two-dimensional (2-D) gels of serum proteins. *a*, Purified MOPC-21 immunoglobulin (Littion Bionetics). *b* and *c*, 25 μ l of serum of + and - offspring A, respectively. *d*, Additional examples of the characteristic 2-D gel regions of serum from one transgenic mouse (+) and five normal littermates (-). The sera were precipitated with 40% ammonium sulphate, the precipitate redissolved in glass distilled water and run on 2-D gels after O'Farrell gels which were silver stained³⁵. Molecular weights (MW) of marker proteins run in the second dimension of the same gel (*a*) or of a control gel (*b*, *c*) are indicated.

not express the injected gene. This indicates that immunoglobulin gene rearrangement is not the sole required event for the tissue-specific expression of the genes by B cells. Rather, signals necessary for tissue-specific expression must be contained within the 14.8 kb of injected pB1-14 DNA. Other studies have suggested a possible immunoglobulin gene control region. The observations that unrearranged C_{κ} genes, but not generally V_{κ} genes, are DNase I sensitive¹⁹, undermethylated²⁰ and transcribed²¹ in B-lymphoid cell lines indicate that some control region other than the 5' flanking region of the rearranged gene

is probably associated with C_{κ} . A short region within the $J_{\kappa}5-C_{\kappa}$ intron, which is highly conserved between mouse and man²² and which is DNase I hypersensitive in activated B lymphocytes²³ may be the tissue-specific control sequence. Also, this portion of the $J_{\kappa}-C_{\kappa}$ intron seems to be important for expression of κ genes transfected into lymphoid cells²⁴⁻²⁶. An analogous sequence is present in immunoglobulin heavy-chain genes²⁷⁻²⁹. We are presently determining if the sequence within the $J_{\kappa}-C_{\kappa}$ intron is responsible for tissue-specific expression of κ genes in transgenic mice.

B cells rearrange their heavy-chain locus before their light-chain loci³⁰. For the first time in these transgenic mice, developing B cells have a functionally rearranged light-chain gene present before heavy chain genes are rearranged. It will be interesting to investigate the possible effects of this situation on B-cell ontogeny, with respect to the rearrangement and regulation of endogenous genes.

Because the migration of the protein from the injected gene is identical on 2-D gels to the purified protein from MOPC-21, it can be inferred that the coding and control sequences have remained intact and that the protein is properly translated, glycosylated and secreted in transgenic mice.

The technology of microinjection of cloned genes into fertilized mouse eggs has been proven to be a powerful tool for the introduction of fusion genes with the ubiquitously expressed metallothionein promoter. The immunoglobulin κ gene is a clear example where a natural gene and one which is normally expressed in a highly tissue-specific manner apparently retains this property in transgenic mice. The details of the control of κ gene expression can now be studied in B-cell hybridomas obtained from these transgenic mice.

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Different promoters for the *Anabaena* glutamine synthetase gene during growth using molecular or fixed nitrogen

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*The major RNA products of the cyanobacterium (blue-green alga) *Anabaena* glutamine synthetase (glnA) gene are transcribed from different promoters during growth utilizing molecular or fixed nitrogen. During growth utilizing ammonia the promoter sequence resembles the consensus sequence of *Escherichia coli* promoters; in nitrogen-fixing conditions the promoter sequence resembles those of *Anabaena* nitrogen-fixing (nif) genes.*

GLUTAMINE synthetase (coded by the *glnA* gene) is a key enzyme in nitrogen assimilation in all organisms. In the heterocyst-forming cyanobacterium *Anabaena* 7120, nitrogen reduced to ammonia by nitrogenase is incorporated into glutamine by glutamine synthetase. The glutamine is then transported into the vegetative cells to serve in the biosynthesis of various metabolites. Glutamine synthetase is needed whether *Anabaena* cells are growing on ammonia or fixing nitrogen; thus the enzyme is present in the vegetative cells as well as in the heterocysts¹.

The *Anabaena glnA* gene is of additional interest because its product, glutamine synthetase, is important in the symbiotic association of *Anabaena* with the water fern *Azolla*. In that association, *Anabaena* nitrogen fixation supplies all the nitrogen requirements of the host plant². Glutamine synthetase activity is very low in the symbiont, as is the level of *Anabaena* glutamine synthetase antigen³. The host plant, therefore, appears to regulate synthesis of glutamine synthetase in the symbiont. By doing so, the product of nitrogen fixation in the symbiont is principally ammonia, which is exported by the symbiont and taken up by the host.

Studies with a variety of organisms have shown that glutamine synthetase can be regulated at the level of both enzyme activity and enzyme synthesis. In the enterobacteriaceae, glutamine synthetase biosynthetic activity is switched off by the covalent addition of AMP to a tyrosine residue on each of the enzyme's 12 subunits⁴. The adenylation system responds to the ratio of glutamine to α -ketoglutarate in the cell, which affects the adenylation system through a cascade of other enzyme modifications. The bacillaceae, and cyanobacteria such as *Anabaena*, do not have the adenylation cascade system, but instead appear to regulate glutamine synthetase activity by additive feedback inhibition mediated by several amino acids^{3,5}. The product of the cloned *glnA* gene of *Anabaena* is not adenylated in an *Escherichia coli* strain capable of full adenylation of the product of a cloned *E. coli glnA* gene⁶. This result has been interpreted to mean that the *Anabaena* enzyme lacks the site for modification by the adenylation system.

The regulatory circuits controlling transcription of the structural gene for glutamine synthetase, *glnA*, and the genes for nitrogen fixation, *nif*, in *Klebsiella pneumoniae* have been found to intersect in remarkable ways^{7,8}. High-level transcription of both *glnA* and *nifA* requires activation by the product of the *ntrC* gene which, in *E. coli* and *Salmonella* as well as in *Klebsiella*, maps near *glnA*⁹⁻¹¹. The *nifA* gene product is, in turn, required to activate transcription of the other *nif* genes¹². The *nifA* and *ntrC* gene products are probably related evolutionarily, since under certain circumstances *ntrC* can replace *nifA* in *nif* gene activation and *nifA* can replace *ntrC* in *glnA* gene activation^{7,8}. These symmetries suggest that DNA sequences in the promoter regions of the *glnA* and *nif* genes may be related

and, indeed, similarities in the 5'-flanking sequences of these genes have been noted¹³.

The gene coding for glutamine synthetase in the heterocyst-forming cyanobacterium *Anabaena* 7120 has been cloned⁶. That gene is expressed in *E. coli* at a level that is comparable with an activated *E. coli glnA* gene, independent of the concentration of ammonia in the growth medium, and without a requirement for the *ntrC* gene product⁶. In *Anabaena* itself, the gene is expressed at a slightly higher level in nitrogen-starvation conditions (derepressing for nitrogenase) than in ammonia-containing medium³.

Several of the genes for nitrogen fixation in *Anabaena* have been cloned and sequenced¹⁴⁻¹⁷ and the origins of their transcripts have been determined by S₁ nuclease mapping and primer extension (S.J.R. and R.H., in preparation). Examination of the DNA sequences in the -10 and -35 regions of these genes revealed a consensus for *Anabaena nif* gene promoters that differs both from the *nif* gene promoters of *Klebsiella* and from the conventional promoters of *E. coli* and *Bacillus subtilis* (S.J.R. and R.H., in preparation). Transcripts corresponding to these genes are found in *Anabaena* only in conditions in which nitrogenase is derepressed¹⁸. Since glutamine synthetase is present in *Anabaena* in both nitrogenase repressing and derepressing conditions, it was of interest to determine the origins of the *glnA* transcripts produced in both conditions.

Nucleotide sequence of *glnA* gene

To determine the promoter sequences and to characterize the structural features of the glutamine synthetase gene, the cloned DNA fragment coding for *Anabaena glnA* was sequenced by the procedure of Maxam and Gilbert¹⁹. The restriction map of the fragments used for sequencing and the sequencing strategy are shown in Fig 1. The entire 1,425-base pair (bp) coding sequence, along with 380 bp of 5' flanking and 420 bp of 3' flanking sequence, is presented in Fig. 2, along with the amino

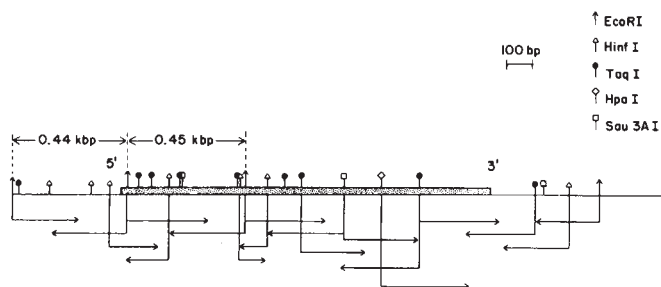


Fig. 1 Restriction endonuclease map and sequencing strategies for the *glnA* gene of *Anabaena* 7120. Only the restriction sites used in sequencing are indicated. The arrows indicate the direction of sequencing and the lengths of the sequences obtained.

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GCTTGTACT										GCATCGCGCA									
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TCCCAATCTT										TGCTATCTAT									
GGTTTGATAT										-250									
TCTGTTACTT										GTTAATATTA									
TGTTGTCATCA										AGGAGTAACA									
TAATTTGGTT										GCACTACGCA									
AAGGATTTTA										-150									
TGTCAAAGTT										GACCCCTATG									
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CCTGTAATCC										TAACAAAGAC									
TACAAAACCTG										-100									
TCTAATGTTT										AGAACTCAGG									
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TCTGGTTTGA										ATTTAAGAAA									
TTTGGCAGAT										TTGGCAGAGA									
TCTGGTTTGA										ATTTAAGAAA									
TTTGGCAGAT										TTGGCAGAGA									
TCTGGTTTGA										ATTTAAGAAA									
TTTGGCAGAT										TTGGCAGAGA									
TCTGGTTTGA										ATTTAAGAAA									
TTTGGCAGAT										TTGGCAGAGA									
TCTGGTTTGA										ATTTAAGAAA									
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TTTGGCAGAT										TTGGCAGAGA									
TCTGGTTTGA										ATTTAAGAAA									
TTTGGCAGAT										TTGGCAGAGA									
TCT																			

Fig. 2 Nucleotide sequence of the *glnA* gene of *Anabaena* 7120. The amino acid sequence of glutamine synthetase predicted from the nucleotide sequence is printed below the nucleotide sequence. The sequence of the non-coding strand is shown. The amino acid residues are numbered on the right. The origins of transcription of N⁻ RNA are shown with solid arrows (below) and the origins of transcription of N⁺ RNA are shown by open arrows (above). The -10 and -35 sequences before the start sites of RNA_I, RNA_{II}, and RNA_{IV} and the putative ribosome-binding sequence are underlined. The start sites of RNA_{III} are shown with smaller arrows.

Methods: DNA was sequenced by the method of Maxam and Gilbert¹⁹ except for the piperidine cleavage step which was performed as described by Smith and Calvo³¹. Both strands were sequenced and all the restriction sites were overlapped. The modified DNAs were analysed on 5%, 8% and 20% polyacrylamide gels as described¹⁹ and autoradiographed with Kodak XAR-5 film using intensifier screens. Sequencing ladders were entered into an Amdahl computer and translated using the program of Queen and Korn³².

acid sequence predicted from the nucleotide sequence. Using this sequence as a guide, it has been possible to locate, by homology, most of the sequenced peptides of the enzyme from *E. coli* (L. M. Keefer and R. L. Heinrikson, manuscript in preparation). Of particular interest is the sequence around Tyr 402, which corresponds to the site of adenylation in the *E. coli* enzyme²⁰. Since *Anabaena* also contains Tyr at that position, its inability to be adenylation in *E. coli* must be due to structural differences rather than to the absence of the target amino acid.

Glutamine synthetase purified from *Anabaena* has an N-terminal amino acid sequence that begins with threonine²¹. Glutamine synthetase was also purified from *E. coli* ET8051 (*E. coli* K-12 Δ (*rha-glnA*)) transformed with a plasmid containing the cloned *Anabaena glnA* gene (pAn503)⁶ and analysed by automated Edman degradation. The protein sequence thus obtained also begins with Thr and agrees perfectly with that predicted by the gene sequence up to residue 30, at which point the sequenator run was terminated.

Five nucleotides upstream from the ATG where translation begins is AGGAG which is complementary to the sequence at the 3' end of *E. coli* 16S rRNA. The presence of a good ribosome-binding sequence upstream from the initiator methionine could be responsible in part for the high level of expression of *Anabaena* glutamine synthetase in *E. coli*⁶.

Origins of transcription of *glnA* gene

To study the origins of transcription of the *glnA* gene in *Anabaena*, RNA was prepared from cells grown in two different conditions. One culture was grown on medium containing 2.5 mM ammonium sulphate (N⁻). A similar culture was washed and resuspended in ammonia-free medium. The herbicide DCMU (which inhibits the O₂-evolving photosystem II) was added and the cells were bubbled with argon for 10 h. These conditions lead to abundant derepression of the *nif* genes (N⁻)²². The two RNA preparations were fractionated by electrophoresis on agarose gels, blotted onto nitrocellulose paper, and probed with the 0.45 kbp *EcoRI* fragment of pAn503 (Fig. 3)²³. In both cases the preponderant message species was about 1.5 kb, corresponding roughly to the coding length required for the gene (1.4 kb, Fig. 2). The intensity of hybridization was higher with N⁻ RNA, indicating that *glnA* message was more abundant in conditions leading to the derepression of the *nif* genes. This result agrees with the physiological observation that glutamine synthetase activity doubles when *Anabaena* is transferred to nitrogen-free media³.

The two RNA preparations were then used for S₁ nuclease protection²⁴ and primer extension²⁵ experiments. The 0.44 kbp *EcoRI* fragment, which contained 5' flanking sequences and 33 bp of coding sequence, was labelled at its 5' ends, the strands

Fig. 3 Northern blot hybridization indicates the presence of *Anabaena glnA* message in both ammonia-grown (N^+) and nitrogen-fixing (N^-) cells. In both cases, the predominant band corresponds to a message size of 1.5 kb.

Methods: Cultures of *Anabaena* 7120 were grown in Kratz and Myers³³ medium supplemented with 2.5 mM $(NH_4)_2SO_4$. At late log phase, cells were collected by centrifugation, washed repeatedly, then resuspended in fresh medium either with (N^+) or without (N^-) ammonia, as above. After bubbling for 10 h with air/0.5% CO_2 , these cultures were then purged with argon for an additional 10 h. In the N^- culture, 20 μ M DCMU was added during argon purging to inhibit oxygenic photosynthesis and thereby provide complete anaerobiosis. Cells thus ammonia-starved anaerobically are capable of nitrogen fixation²²; this was confirmed by assays of acetylene reduction before RNA isolation. To isolate RNA, cultures were purged with argon while the cells were collected by centrifugation in a continuous flow rotor. The cell pellets were resuspended to a volume of 5 ml in TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) then briefly shaken on ice with 1 ml chloroform. The cells were then disrupted by vortexing with glass beads (0.45 mm diameter). After repeated extraction with phenol/chloroform/ether (50:50:1), the aqueous phase was adjusted to 0.3 M sodium acetate, from which the nucleic acids were ethanol-precipitated. The nucleic acid pellet was resuspended in TE, and the RNA purified by pelleting twice through CsCl gradients³⁴. Aliquots containing 20 μ g of N^+ or N^- RNA were denatured with dimethyl sulphoxide and glyoxal³⁵, then fractionated on 1% agarose gels. Size markers, consisting of DNA restriction fragments of known lengths, were similarly denatured and fractionated, then stained with acridine orange; sizes are shown at right in the figure. The RNA was transferred to nitrocellulose paper²³, then hybridized to the 0.45 kbp internal *EcoRI* fragment of the *Anabaena glnA* gene (Fig. 1), which had been labelled by nick translation.

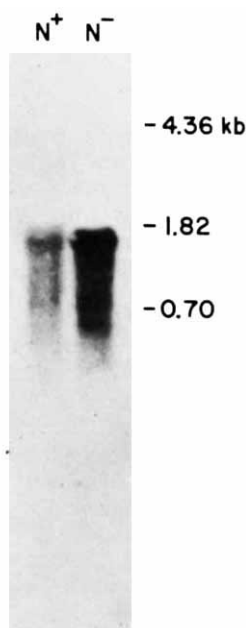
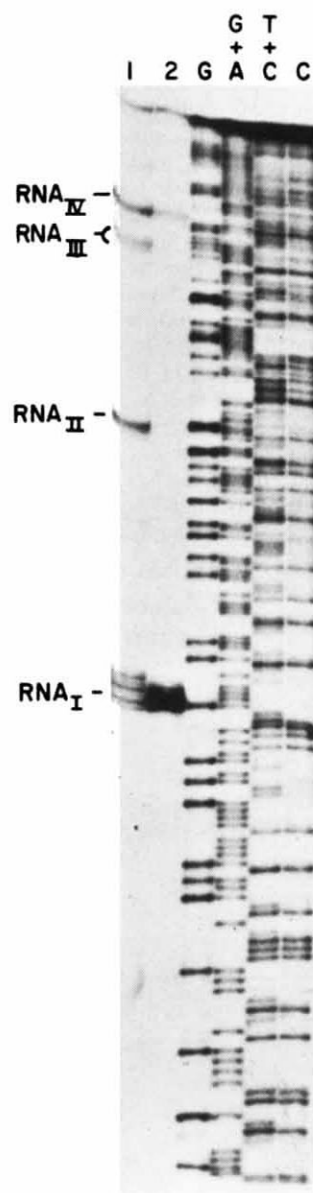


Fig. 4 Identification of the start points for glutamine synthetase transcription in the presence (N^+) or absence (N^-) of ammonia in the growth medium. The protected fragments are shown along with the sequencing reaction products of the 0.44 kbp *EcoRI* fragment in lanes G, G + A, T + C, and C. Lane 1, 0.44 kbp *EcoRI* fragment hybridized with N^+ RNA and digested with 50 units of S_1 nuclease. Lane 2, 0.44 kbp *EcoRI* fragment hybridized with N^- RNA and digested with 50 units of S_1 nuclease.

Methods: The 0.44 kbp *EcoRI* fragment at the 5' end of the coding region was labelled at its 5' ends, the strands were separated and the coding strand (20,000 c.p.m.) was hybridized with 130 μ g of total *Anabaena* RNA isolated in N^+ or N^- conditions. Hybridization was performed in 50 μ l of 80% deionized formamide, 0.4 M NaCl, 0.04 M MES, pH 6.4 and 1 mM EDTA²⁴. After heating at 65 °C for 10 min, the reactions were incubated at 37 °C for 4 h. The samples were then diluted 10-fold with 0.28 M NaCl, 0.03 M NaOAc pH 4.6, 4.5 mM $ZnSO_4$ containing 50 units of S_1 and incubated at 37 °C for 1 h. Twenty μ g of tRNA was added and the nucleic acids were precipitated with EtOH. In titration experiments where the amount of N^+ RNA was varied from 0.6 μ g to 150 μ g, no differences in the relative intensity of the bands corresponding to RNA_I and RNA_{II} were detected. Intensity of both increased as the RNA concentration was raised, indicating that S_1 protection experiments were done in conditions of DNA excess.



were separated and the coding strand was hybridized with RNA from N^+ or N^- cells. The RNA-DNA hybrids were treated with S_1 nuclease and electrophoresed, along with sequencing cleavage products of the 0.44 bp *EcoRI* fragment. The results show that the same family of bands (RNA_I) are produced by both preparations of RNA (Fig. 4). A family of bands representing a longer RNA (RNA_{II}) were produced only with N^+ RNA but not with N^- RNA. In addition, two groups of bands representing larger RNAs (RNA_{III} and RNA_{IV}) were produced with N^+ RNA, only one of which was produced with N^- RNA (RNA_{IV}). The starts of transcription were determined from the position of the major band in each family. These are indicated in Fig. 2 where the solid arrows (below) indicate the *glnA* origins used in *nif* derepressing (N^-) conditions and the open arrows (above) indicate the origins used in ammonia-containing media. RNA_I, present in N^+ and N^- conditions, starts at position -93, while RNA_{II}, present only in the N^+ condition, starts at position -155. The two bands corresponding to RNA_{III} map at positions -244 and -247, while RNA_{IV} maps at position -267.

These start points of transcription were confirmed by primer extension. To prepare the primer, the 0.44 kbp *EcoRI* fragment was labelled at its 5' ends, digested with *DdeI*, and the 88 bp *DdeI-EcoRI* fragment that lies between positions -55 and +33 was strand-separated. The labelled strand was then hybridized

with N^+ or N^- RNA and extended using avian myeloblastosis virus (AMV) reverse transcriptase. The extended primers are shown along with the S_1 protected fragments in Fig. 5. There is excellent agreement between the two methods for mapping RNA_I and RNA_{II}. The fainter bands which are smaller than these two RNA classes are usually observed in primer extension experiments and could be due to pauses during reverse transcription. However, although RNA_{III} and RNA_{IV} are evident in the S_1 experiments, they are not visible in the primer extension experiments using the *DdeI-EcoRI* primer. A primer was therefore generated from a region further upstream (*HinfI-DdeI* fragment which lies between positions -105 and -65) and reverse transcribed. In this case, fragments corresponding to RNA_{III} and RNA_{IV} are observed (Fig. 6), along with RNA_{II}.

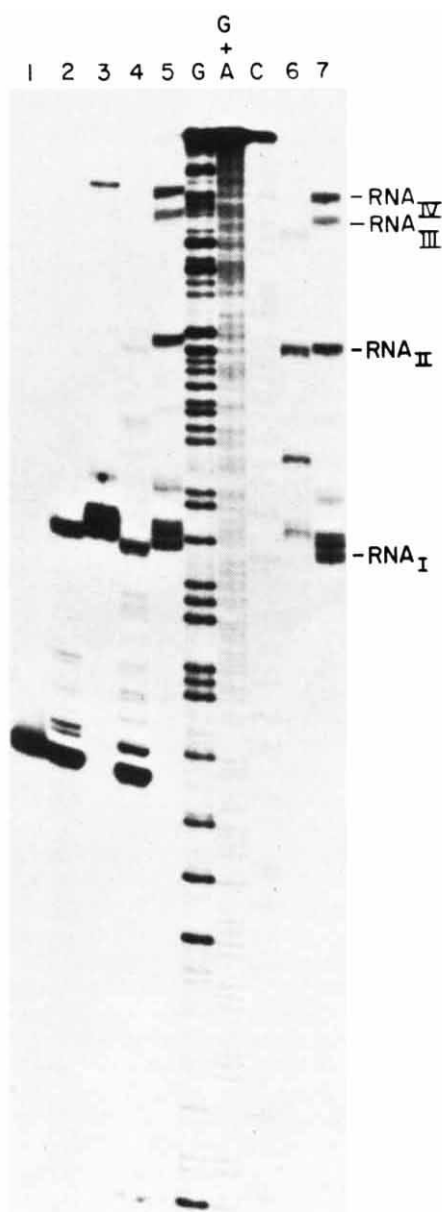
The primer extension results agree with S_1 -mapping and confirm that RNA_{II} is present only in N^+ conditions, but that the shorter transcript (RNA_I) is present in both N^+ and N^- conditions. Additional longer transcripts (RNA_{III} and RNA_{IV}) appear in both conditions.

The sequences corresponding to -10 and -35 for each origin are underlined in Fig. 2, and compared with various other prokaryotic promoter sequences in Table 1. The promoter for RNA_{II}, utilized only during growth on ammonia, looks like a typical *E. coli* promoter²⁶. In contrast, the promoter sequence

for RNA_I is quite unlike the *E. coli* consensus sequence, but compares well with the promoter sequences of *Anabaena nif* genes whose transcripts have been mapped (S.J.R. and R.H., in preparation). *Nif* gene transcripts are found in *Anabaena* only in conditions derepressing for nitrogenase; that is, anaerobic and ammonia-free¹⁸. In these conditions, *glnA* gene transcription from the downstream *nif*-like promoter is increased, while transcription from the upstream *E. coli*-like promoter is abolished (Figs 4–6). Neither *E. coli*-like nor *nif*-like promoter sequences appear upstream of the two similarly sized transcripts at RNA_{III}. Instead, the –10 region for these transcripts is the end of a 9-base inverted repeat which could form a stem-loop structure (from position –258 to –292). The significance of this possible secondary structure is unclear, as is the meaning of the apparent transcription starts at RNA_{III}. RNA_{IV}, present in both N⁺ and N[–] grown cells, has a promoter composed of *E. coli*-like –10 and *Anabaena nif*-like –35 sequences (Fig. 2). Neither RNA_{III} nor RNA_{IV} show a consistent pattern of variation in abundance in ammonia-grown or nitrogen-fixing conditions, whereas RNA_{II} disappears and RNA_I increases in abundance in conditions of nitrogen fixation.

Transcription of *Anabaena glnA* in *E. coli*

The *Anabaena glnA* gene is expressed in *E. coli*, independent of the *ntnC* gene product and unregulated by ammonia, using



a promoter within the cloned *Anabaena* DNA fragment⁶. RNA prepared from *E. coli* HB101 carrying pAn503, grown in the presence of ammonia, was used for S₁ nuclease protection, as shown in Fig. 5. The major transcript originates at the same position as RNA_{II} observed in ammonia-grown *Anabaena*, that is, it uses the *E. coli*-like promoter identified before the open arrow at position –155 in Fig. 2. In addition, there is a less abundant transcript that maps at position –114. There are no appropriate *E. coli*-like –10 and –35 sequences upstream from this position. This transcript could arise from the longer major transcript by a processing event. Possible stem-loop structures that support this suggestion are observed upstream from the region where the second transcript initiates. Bands corresponding to RNA_I, RNA_{III} and RNA_{IV} are not seen.

Conclusions

Using Northern hybridization, we have observed the following pattern of transcription in *Anabaena*: transcripts corresponding to two genes with *nif* promoters are absent from ammonia-grown cells but present abundantly in anaerobic, ammonia-starved cells, while transcripts corresponding to the gene for the large subunit of ribulose biphosphate carboxylase are abundant in ammonia-grown cells but absent from ammonia-starved cells¹⁸. As seen in Fig. 3, the *glnA* transcripts appear in both ammonia-grown and ammonia-starved cells. However, S₁ mapping and primer extension show that these transcripts are initiated from different promoters. In ammonia-grown cells, both *E. coli*-like and *nif*-like promoters are used. In ammonia-starved cells, nearly all the transcripts are initiated at the *nif*-like promoter; there appears to be no transcription from the *E. coli*-like promoter. Under both conditions, there are additional transcripts of greater size. The sequence at the –10 region of the *nif*-like promoter of RNA_I is TCTAC and the –35 region is CAAAAC (Table 1). These sequences are very different from *E. coli*-like promoter sequences²⁶. However, they are highly similar to the –10 and –35 regions of the *Anabaena nif H*

Fig. 5 Comparison of the origins of *glnA* transcription in *Anabaena* 7120 and in *E. coli*. The 5' ends of glutamine synthetase mRNA determined by S₁ nuclease protection were confirmed by primer extension with AMV reverse transcriptase. Lane 1, the 88 bp *DdeI*–*EcoRI* fragment between positions –55 and +33. Lane 2, extension of the 88 bp primer annealed to N[–] RNA. Lane 3, S₁ nuclease protected fragments of the 0.44 kbp *EcoRI* fragment annealed to N[–] RNA. Lane 4, extension of the 88 bp primer annealed to N⁺ RNA. Lane 5, S₁ nuclease protected fragments of the 0.44 kbp *EcoRI* fragment annealed to N⁺ RNA. Lanes G, G + A and C are the cleavage products of the 0.44 kbp *EcoRI* fragment used in the S₁-protection experiment. Lane 6, S₁ nuclease protected fragments of the 0.44 kbp *EcoRI* fragment annealed to RNA from *E. coli* HB101 (pAn503). Lane 7, S₁ nuclease protected fragments of the 0.44 kbp *EcoRI* fragment annealed to N⁺ RNA.

Methods: Primer extension experiments were performed as described by Kassavetis and Geiduschek²⁵. 130 µg of N⁺ or N[–] RNA was hybridized to 20,000 c.p.m. of the 5' end-labelled and strand separated 88 bp primer. 10 units of reverse transcriptase were added to the hybridization mixture and reverse transcription was allowed to proceed for 1 h at 37 °C. The reaction mixture was then extracted with phenol and precipitated twice with ethanol. The primer extended products were dissolved in Maxam and Gilbert sequencing reaction buffer and analysed along with the sequencing reaction products. To map the origin of *Anabaena* glutamine synthetase transcripts in *E. coli*, HB101 (pAn503) was grown at 37 °C in a shaker bath to A₆₀₀ = 0.7 in M9 medium which contains 18 mM NH₄Cl and casamino acids³⁶. The RNA isolation was as described by Kassavetis and Geiduschek²⁵ with the following modifications: after cell lysis and removal of precipitated proteins, 170 µl 98% β-mercaptoethanol and 5.5 g CsCl was added to 11 ml of supernatant. This solution was layered over 2 ml of 5.7 M CsCl in an SW41 tube and spun at 35,000 r.p.m. for 18 h. The RNA pellets were resuspended in TE, precipitated with ethanol and dissolved in the same buffer. For S₁ mapping, 130 µg of *E. coli* RNA was hybridized with 20,000 c.p.m. of 5' end-labelled and strand-separated 0.44 kbp *EcoRI* fragment. Hybridization and S₁ digestion conditions were exactly as described in Fig. 4 legend.

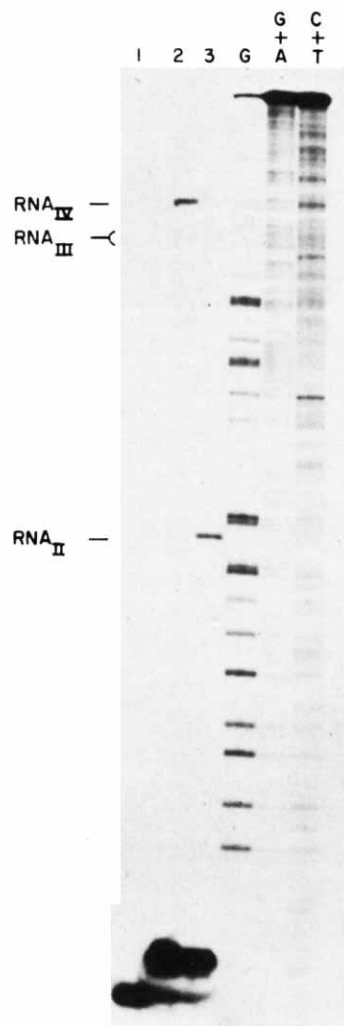


Fig. 6. Detection of RNA_{II}, RNA_{III} and RNA_{IV} by reverse transcription of a primer upstream from the *Anabaena glnA* coding sequence. Lane 1, 40 bp *HindIII-DdeI* fragment between positions -105 and -65. Lane 2, extension of 40 bp primer annealed to N⁻ RNA. Lane 3, extension of 40 bp primer annealed to N⁺ RNA. Lanes G, G + A and C + T are the cleavage products of the *HindIII-DdeI* fragment between positions -380 and -65.

Methods: Primer extension experiments were performed as described in the legend to Fig. 5 except that 300 µg of N⁺ or N⁻ RNA was used in the hybridizations. generate sequencing ladder corresponding to the primer, the *HindIII-DdeI* fragment was strand-separated and the coding strand was subjected to Maxam and Gilbert sequencing reactions¹⁹.

promoter (S.J.R. and R.H., in preparation). This is not unexpected since glutamine synthetase is expressed when cells are fixing nitrogen. The level of expression is greater, indicating that transcription from *glnA* is activated in conditions derepressing for nitrogenase. When *Anabaena* cells are grown in the presence of ammonia there is a lower level of transcription from the *nif*-like promoter as well as transcription from other promoters which are upstream from the *nif*-like promoter. The -10 and -35 sequences upstream from the second transcription start are TAATAT and TTGTGC, respectively. These sequences are similar to *E. coli* promoters²⁶, they are used by the *Anabaena glnA* only when ammonia is present in the growth medium.

A similar situation has been observed in bacilli where several genes have been found to be transcribed from different but overlapping promoters. The *spoVG* gene of *Bacillus subtilis* is transcribed from an upstream promoter by RNA polymerase in which the major vegetative cell σ factor is replaced by a minor σ factor, called σ^{37} . The same gene can also be transcribed from a promoter 10 bp downstream by RNA polymerase with yet another σ factor, termed σ^{32} ; the role of these dual promoters, and the control of *SpoVG* expression by the *spoD* loci, is not understood²⁷. In *Bacillus thuringiensis*, a crystal protein gene is transcribed early in sporulation from a downstream promoter and later in sporulation from another promoter 15 bp upstream²⁸. The -10 and -35 regions of the two crystal protein gene transcripts differ from each other, and from the consensus -10 and -35 sequences for *B. subtilis* vegetative cell genes. In both cases these results have been interpreted in terms of a mechanism for continuing transcription of genes through sporulation, when RNA polymerase is modified by replacement and addition of σ factors^{27,28}. Transcription of the same gene from

Table 1 Comparison of *Anabaena glnA* promoter sequences with other promoters

<i>Anabaena glnA</i> 'nif-like' promoter (RNA _I)	CAAAAC -35	TCTAC -13
<i>Anabaena nifH</i> promoter*	CATAAC -41	TCTAC -14
<i>ntr</i> -regulated consensus promoter†		TTTGCA -17
<i>Anabaena glnA</i> 'E. coli-like' promoter (RNA _{II})	TTGTGC -37	TAATAT -13
<i>E. coli</i> consensus promoter‡	TTGaca -36 to -34	TataaT -14 to -11

* S.J.R. and R. H., in preparation. † Ref. 13. ‡ Ref. 26.

two different promoters, each recognized by a different species of RNA polymerase, had previously been recognized in the programme of transcription of phage SPO1 in *B. subtilis*^{29,30}.

The situation in *Anabaena* differs in that the promoters for the two smaller transcripts (RNA_I and RNA_{II}) are 62 bp apart, and nearly 100 bp distant from the origins of the larger transcripts; distances sufficient for RNA polymerase bound at one site not to interfere with binding at the others. The requirement for multiple promoters would be analogous, however. Glutamine synthetase is needed for ammonia assimilation whether cells are growing on ammonia or fixing nitrogen. If σ NA polymerase is modified for abundant transcription of *nif* genes (as proposed for *Klebsiella*^{7,13}) and for cessation of transcription of genes coding for proteins involved in CO₂ fixation and O₂ evolution, the *glnA* gene has to be transcribed by both forms of the enzyme. Multiple promoters seem a reasonable, simple solution to the problem of ensuring continued expression of *glnA* in the presence or absence of ammonia. It is still necessary to explain how the *nif*-like promoter is used in N⁺ conditions. We suggest that ordinarily such promoters are transcribed only by a modified form of RNA polymerase, which should not be present under N⁺ conditions. However, it is possible that the *nif*-like promoter for RNA_I could also be activated for transcription initiation by transcription through it from the upstream, *E. coli*-like promoter.

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An unusual transposon with long terminal inverted repeats in the sea urchin *Strongylocentrotus purpuratus*

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A 3-kilobase DNA segment characteristic of a transposable element was found within a histone H2B pseudogene in a higher eukaryote, the sea urchin Strongylocentrotus purpuratus. The inserted segment (TU1) is flanked by 8-base pair (bp) direct repeats of the H2B sequence. TU1 has long terminal inverted repeats ~840 bp long with an outer domain of 15-bp tandem repeats and a non-repeating inner domain, and is a member of a heterogeneous family of transposable elements. TU1 differs from most previously characterized eukaryotic transposable elements with terminal direct repeats, but resembles the foldback transposon family in Drosophila.

TRANSPOSABLE genetic elements were first identified in maize¹ and have since been found in bacteria, yeast and insects²⁻⁴. Recent experiments have shown that the provirus forms of both avian and mammalian retroviruses have structural similarities to some classes of transposable elements found in eukaryotic cells^{5,6}. Furthermore, the possible role of transposition has been implicated in oncogenesis^{7,8} and in the speciation of *Drosophila melanogaster*⁹. Moreover, the multiple, sequential and developmentally regulated DNA rearrangements involved in the formation of functional immunoglobulin genes seem to be analogous to the rearrangements mediated by transposable elements¹⁰. Finally, there is evidence that in eukaryotes transposable genetic elements may have a role in the generation of genetic diversity in evolution through mechanisms involving DNA rearrangements¹¹. Such a role for transposable elements has also been suggested in sea urchins, whose genomes are characterized by vast quantitative and qualitative differences between the many families of dispersed repetitive sequences seen even among closely related species¹²⁻¹⁴. We report here the discovery and unusual structural characteristics of a new family of eukaryotic transposable genetic elements that are present in multiple copies in the sea urchin genome.

Insertion sequence in histone H2B gene

The several hundred tandemly repeated copies of histone genes expressed early in sea urchin development have been studied and characterized in detail down to the nucleotide sequence level¹⁵⁻¹⁷. Some of these early histone-coding regions, however,

are not contiguous with the tandemly repeated early genes and are instead dispersed as physically distant fragments termed orphans¹⁸. As shown below, the transposable element, TU1, was discovered following investigations by Childs *et al.*¹⁸ involving screening experiments for these histone-coding sequences embedded in uncharacteristic restriction endonuclease-generated DNA fragments.

In those screening experiments, a mixture of nick-translated histone probes, specific to all five early histone genes, was used to identify λ Charon 4A phage clones that contain histone-specific sequences. Agarose gel electrophoresis of the DNA isolated from approximately 160 such clones following plaque purification and digestion with *EcoRI* endonuclease, revealed about 20 histone gene variants that did not yield the expected 4.6- and 2-kilobase (kb) endonuclease cleavage fragments of the *Strongylocentrotus purpuratus* early histone gene cluster¹⁹; instead, DNA fragments larger or smaller than those expected were seen (data not shown).

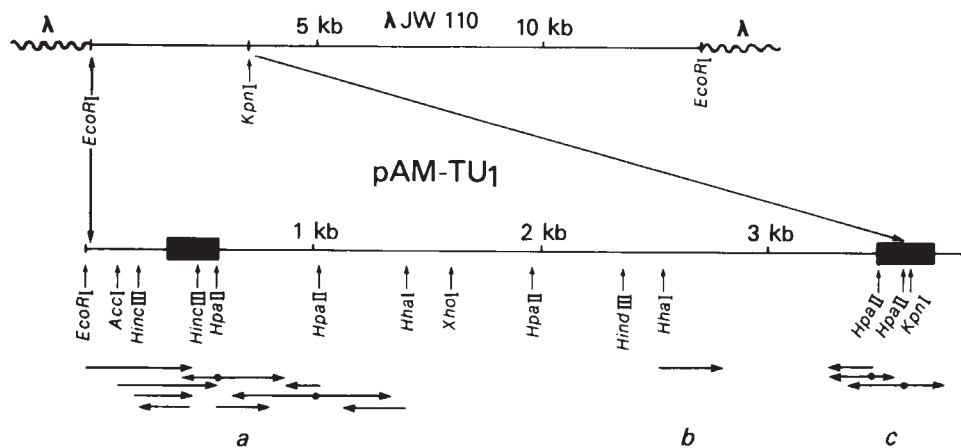
One variant clone, λ JW110, containing a single 13.6-kb *EcoRI*-generated fragment, proved particularly interesting and its analysis is the subject of this report. λ JW110 hybridized to the H2B-specific probe, but not to other histone coding sequences. A restriction endonuclease cleavage map was constructed and the various fragments were tested for homology with the H2B probe; only the 3.6-kb *KpnI-EcoRI* DNA fragment of the 13.6-kb insert hybridized with the probe (Fig. 1). Furthermore, several noncontiguous *HpaII*-generated segments of this fragment hybridized to either one of two DNA probes specific to the 5' or 3' coding region of the H2B gene, implying that the H2B histone coding region was split in the λ JW110 clone by the insertion of non-histone DNA.

As sea urchin histone genes are known not to contain intervening sequences (introns), it seemed possible that the DNA segment interrupting the H2B sequences might be a transposable

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Fig. 1 Restriction endonuclease map of bacteriophage clone λ JW110 containing histone H2B sequences split by the insertion of the transposable element TU1. The upper line represents the 13.6-kb *EcoRI* λ insert. Wavy lines represent the λ arms. The lower line is an expanded map of the 3.6-kb long *EcoRI*–*KpnI* restriction endonuclease DNA digestion fragment. The thick lines represent regions that hybridize to 5'-specific or 3'-specific H2B-coding probes and were identified by Southern transfer hybridization of DNA fragments generated by digestion with the restriction endonuclease *HpaII*. The arrows below the map represent the strategy used to determine the nucleotide sequence of the H2B segments, their junctions with the insert and with non-histone flanking regions, and several segments of the inserted sequence. Each arrow corresponds to a separate sequencing reaction, and the length and direction of each arrow represent the length and direction of the sequence determined. The sequenced regions *a*, *b* and *c* correspond to the nucleotide sequences presented in Fig. 2.

Methods: A mixed preparation of cloned *S. purpuratus* early histone gene sequences in chimaeric plasmids pSp102 (H1, H4 and H2B genes) and pSp117 (H2A and H3 genes)¹⁹ was radiolabelled by nick translation³² and then used to screen about 60,000 phage plaques³³ (the equivalent of a single *S. purpuratus* genome) from a library of genomic DNA²⁷. The phage blots were preincubated in hybridization buffer (50% formamide, 4×SSC, 50 mM sodium phosphate pH 7, and 0.05% each of bovine serum albumin, Ficoll and polyvinylpyrrolidone) for 6 h at 40 °C and thereafter hybridized with 100,000 c.p.m. ml⁻¹ of the mixed radiolabelled preparation of cloned histone gene DNA fragments. Phage blots were then washed in 0.1×SSC, 1% SDS, 50 mM phosphate pH 7 for 3 h and exposed for autoradiography onto prefogged X-ray film (XR5, Kodak). The DNA isolated from hybridizing, purified phage plaques³⁴ was digested with *EcoRI* and the resulting fragments electrophoresed on agarose gels as described previously³⁵. Some (~20) recombinant phage clones, on digestion with *EcoRI*, did not yield the expected 4.6- and 2-kb fragments of the early histone gene cluster in *S. purpuratus*, and one variant clone, λ JW110, contained a single 13.6-kb *EcoRI* fragment as indicated in the figure. DNA from this variant, isolated as described previously³⁶, was nick-translated and hybridized to a blot³⁷ of electrophoretically resolved fragments of early histone gene DNA, each known to contain one of the five histone-coding sequences. Only the H2B-coding fragment showed hybridization. Using the various restriction enzymes (New England BioLabs) indicated in the figure, a restriction map was generated. We used 5' (pSp2B-4) and 3' (pSp2B-3) H2B-coding region-specific probes³⁸ in conjunction with Southern blots to locate the H2B sequences within several noncontiguous *HpaII*-generated segments of the 3.6-kb *KpnI*–*EcoRI* DNA fragment as indicated. At this stage the 13.6-kb *EcoRI* DNA fragment was subcloned for further studies into the *EcoRI* site of the plasmid pBR322 and the resulting chimaeric plasmid is designated pJW110. The 3.6-kb *KpnI*–*EcoRI*-generated fragment was used to replace a similar fragment in a pBR322–simian virus 40 derivative³⁹ to give the subclone designated pAM-TU1.



genetic element that had inserted itself within the H2B-coding sequences. The following data established that the DNA segment interrupting the H2B sequences is a transposable element which we have termed TU1.

Histone gene sequences

DNA sequence analysis of the 3.6-kb *EcoRI*–*KpnI*-generated fragment was carried out according to the strategy shown in Fig. 1. The nucleotide sequences are presented in Fig. 2; the sequences corresponding to the H2B gene are shaded. The histone mRNA leader sequence 5' to the translation initiation codon for the H2B protein (Fig. 2*a*) starts 7 base pairs (bp) downstream from the known cap site of the authentic early H2B gene (5' end of the shaded sequence). The junction between preserved H2B sequences and the non-histone flanking sequence 3' to the histone gene region is located at a site corresponding to the 3' terminus of the authentic H2B mRNA (Fig. 2*c*). More importantly, immediately downstream from this junction, and within the nonhistone segment of pJW110, is a 32-bp sequence that encodes poly(A). A shorter sequence ATGTGT (see boxed regions in Fig. 2*a*, *c*) immediately flanks the 5' terminus of the H2B mRNA sequence and is repeated just beyond the poly(A) tract located at the 3' end of the coding sequence.

The presence of a poly(A) stretch and the flanking direct repeats strongly suggest that the H2B sequence analysed here has resulted from insertion into the genome of a polyadenylated mRNA species via a reverse transcription step and that the sequence thus represents a processed pseudogene²⁰. However, the molecular basis for such an occurrence is unclear, as no polyadenylated early histone gene transcripts have been reported in the sea urchin¹⁷. Interestingly, at the very 3' end of the mRNA-encoding sequence of the authentic H2B gene there is a sequence AATAA, that does not appear in the other sea urchin histone genes²¹ nor in this H2B pseudogene. This short sequence has characteristics associated with polyadenylation

signals²²; potentially, this site might initiate occasional polyadenylation of transcripts encoded by the authentic H2B gene.

Our nucleotide sequence analysis also indicates that the interrupted H2B DNA sequence shows >99% homology with the previously determined²¹ corresponding sequence of the authentic H2B genes within the tandemly linked histone gene cluster. Of the 568 bases in pAM-TU1 that are homologous to an H2B gene, there are only five changes; two occur in the 5' untranslated leader region, two in the coding region and one in the 3' untranslated trailer region. Both of the coding region changes have occurred through substitution of an isocoding nucleotide in the third codon position, yielding the same amino acid.

TU1 sequences

In both eukaryotes and prokaryotes, insertion of transposable elements usually results in the duplication of a short sequence of the recipient DNA present at the insertion site, thus creating direct repeats—of recipient DNA sequences—flanking the element. Accordingly, we determined the nucleotide sequences at both junctions of the inserted DNA segment and the interrupted H2B-coding sequence, and found that an 8-bp segment of the H2B sequence was duplicated as a direct repeat, CCATGGTC (Fig. 2*a*, *c*). Thus, the insertion of TU1 resulted in duplication of codons corresponding to amino acids 56–58 of the *S. purpuratus* early H2B gene²¹.

The nucleotide sequence data at the TU1–H2B insertion junctions shows that TU1 has terminal inverted repeat sequences (Fig. 2*a*, *c*). To obtain information about the overall structure of the element and an approximate length for the inverted repeat, we carried out electron microscopic analysis of snap-back structures of single-stranded DNA obtained after denaturation-renaturation of the 3.6-kb *KpnI*–*EcoRI*-generated fragment that contains both TU1 and the split H2B pseudogene. The photomicrographs (Fig. 3) reveal that TU1 has terminal inverted repeat segments ~0.84 kb long and a middle sequence ~1.2 kb

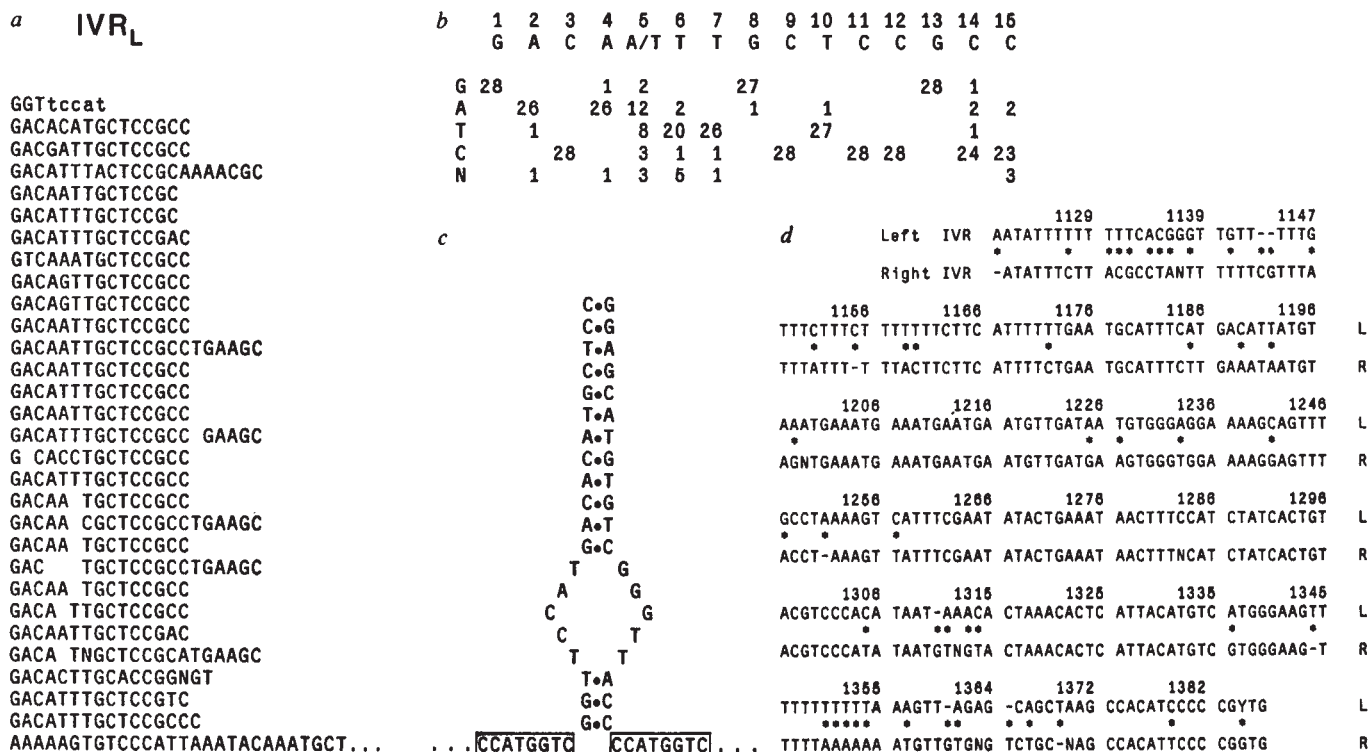


Fig. 4 Nucleotide sequences of selected regions of TU1. **a**, The sequences of the outer domain (OD) of the left inverted repeat segment (IVR_L) are arranged to display their internally repetitious structure. Except for the 5-base nonhomology at bases 4–8 (shown in lower case) of the two IVRs, the IVRs are identical for at least the first 99 bases. These 5-base segments allow us to define and identify a left and right IVR as IVR_L containing the sequence TCCAT, and IVR_R containing the sequence AACCC. **b**, The canonical sequence of the internally repetitive outer domain of the IVR was derived by scoring the base assignment at every position as shown. **c**, The secondary structure formed by one strand of TU1 after it is allowed to snap back. The 8-bp repeat of the H2B-coding sequences is boxed and the potential base-paired and 5-base keyhole loop-out structures are shown. **d**, Analogous sequences of the inner domains of IVR_L and IVR_R were aligned by the IntelliGenetics implementation of the Smith–Waterman modification⁴³ of the Needleman–Wunsch algorithm⁴⁴. The IVR_L sequences represent those from positions 1,120 to 1,387 in sequence **a** in Fig. 2. The IVR_R are the complementary sequences to those shown in sequence **b** of Fig. 2. The alignment was performed with the following parameter settings: SegmentSize 60; Overlap 18; GapPenalty 1; GapSizePenalty 0.333.

the H2B sequence is boxed; the 5-bp nonhomologous region, which we refer to as the 'keyhole', is seen. The keyhole allows us to distinguish and arbitrarily designate a left IVR (IVR_L) containing the keyhole sequence 5'-TCCAT-3' and a right IVR (IVR_R), containing the keyhole sequence 5'-AACCC-3'. With the exception of the keyhole, the sequences of the very ends of both IVRs are identical for at least the first 100 bp (Fig. 2a, c). However, the occurrence of several non-symmetrically located restriction endonuclease cleavage sites within the 840-bp long IVRs (data not shown) suggest that the more internal regions of the IVRs are more heterogeneous. In fact, an alignment of the analogous ID regions of IVR_L and IVR_R (Fig. 4d) indicates that the sequence of these regions is highly conserved in the two IVRs, but it contains numerous single base changes. Figure 5 is a composite map of TU1 based on the results of restriction endonuclease mapping, electron microscopy and nucleotide sequencing.

Multiple copies in sea urchin genome

Transposable elements in *D. melanogaster* and yeast are found to be integrated at different sites in the genome in individual strains of the same species^{24–26}. To determine whether the location of TU1 varied in different individuals, we used probes specific to the TU1 element as well as to the flanking H2B-coding region to screen a λ phage library derived from the genomic DNA of a second *S. purpuratus* individual (supplied by E. Davidson). The second *S. purpuratus* library was estimated to contain essentially all of the single copy sequences of the *S. purpuratus* genome (E. Davidson, personal communication and ref. 27). Although we screened more than 360,000 plaques, equivalent to six genomes, we were unable to detect a bac-

teriophage clone that contained both H2B sequences and sequences homologous to TU1. We thus conclude that the association of TU1 sequences with H2B coding regions is rare; except for the possibility that the second *S. purpuratus* genomic DNA library is incomplete, we can conclude that the insertion site of TU1 in the H2B pseudogene of the individual in which it was detected did not occur in the second individual. We did however, detect numerous λ plaques carrying sequences homologous to TU1 and thus conclude that TU1 sequences are members of a dispersed multigene family.

We next attempt to define the genomic organization of the multiple, dispersed TU1-like elements we had detected in the sea urchin genome. We used fragments from the outer and inner domain of the IVR as well as from the middle of TU1 as radiolabelled hybridization probes to screen sea urchin DNA cut with the restriction endonuclease *EcoRI* (which does not cut within TU1). Autoradiographs of the resulting blots (Fig. 6a) confirm that multiple copies (estimated to be 200–400) of TU1 IVR exist in the sea urchin genome and that the copies do not reside in identically sized *EcoRI*-generated DNA fragments. These results also indicate that the members of the TU1 element family have a highly heterogeneous structure, as different patterns of hybridization were obtained when different segments of TU1 were used as probes (compare the three panels in Fig. 6a). This was further demonstrated by the Southern transfer experiment shown in Fig. 6b, in which sea urchin DNA was cleaved with *HpaII* endonuclease, which cuts within TU1, and an internal *HpaII*-generated fragment of TU1 DNA was then used as the hybridization probe. The resulting autoradiograph revealed a complex series of hybridization bands, leading us to conclude that the TU1 family of sequences is highly heterogeneous.

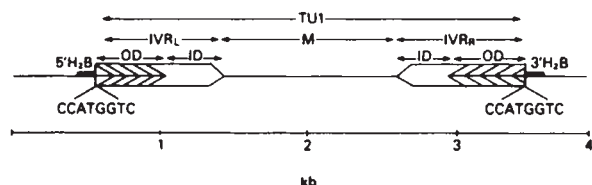


Fig. 5 Schematic summary diagram of the structure of TU1. The large open arrows represent the terminal long IVRs with their outer (OD) and inner (ID) domains demarcated. The middle segment (M) lies between the IVRs. The internally repetitive nature of the OD is represented by the arrowheads. The 8-bp direct repeat of the 'host' DNA is indicated. The scale is in kb.

Discussion

The article describes the discovery of an unusual eukaryotic transposable element, TU1, in a species of higher eukaryote, a deuterostome, the sea urchin *S. purpuratus*. TU1, which appears to be representative of a dispersed multicopy family of such elements in the sea urchin genome, has several distinctive features, including inverted repeat termini that include a short tandemly repeated sequence, plus a middle segment—heterogeneous in length and sequence—between the inverted termini. Mobile genetic elements have not been reported previously in sea urchins, but their existence has been suggested as an explanation for the vast quantitative and qualitative differences observed in many of the dispersed repetitive sequence families seen even between closely related sea urchin species¹²⁻¹⁴ as well as for the postulated horizontal gene transfer of a histone gene cluster between the genomes of two very distantly related sea urchin species²⁸.

The initial copy of a TU1 element was discovered within a histone H2B pseudogene, flanked by an 8-bp direct repeat of the H2B sequences (see diagram of Fig. 5). We assume that this 8-bp duplication of the H2B-coding sequence was generated on insertion of the element within the pseudogene, as duplication of sequences of recipient DNA at the insertion site—yielding flanking direct repeats of the indigenous sequence element—is characteristic of transposable genetic elements in both prokaryotes and eukaryotes²⁻⁴. We predict that analogous 8-bp direct repeats flanking these elements, each having its own unique nucleotide sequence, will be found to bracket other members of the TU1 family. The presence of TU1 within the coding sequence of the H2B pseudogene, the bracketing of the element with direct repeats of the H2B sequence, and our failure to detect a TU element in H2B-coding sequences of another sea urchin individual of the same species support the view that TU1 is a transposable element. More recent experiments (D.L., B.H.-L., S.N.C. and L.H.K., manuscript in preparation) have demonstrated a highly polymorphic genomic organization for TU1 elements from different individuals of the same sea urchin species, collected at the same oceanographic location. In addition, we have isolated from an *S. purpuratus* phage library a number of other elements homologous to TU1, each with characteristics of a transposable element. A detailed analysis of the structures of these TU family members and their differences will be reported elsewhere (B.H.-L., D.L., L.H.K. and S.N.C., manuscript in preparation).

While the structural features of TU1 differ from those of most other known eukaryotic transposable elements which terminate with long direct repeats and have a uniform construction, they share striking similarities with the 'foldback' elements of *D. melanogaster*^{23,29,30} which were described while this work was in progress. The foldback elements are also a dispersed repetitive family of structurally heterogeneous elements having long terminal inverted repeats with an internal direct repeating structure. However, despite the overall structural similarities between the foldback elements and the TU1 family, computer comparison of the DNA sequences of TU1 with the published sequence of members of the *Drosophila* foldback family has failed to identify any significant homologies.

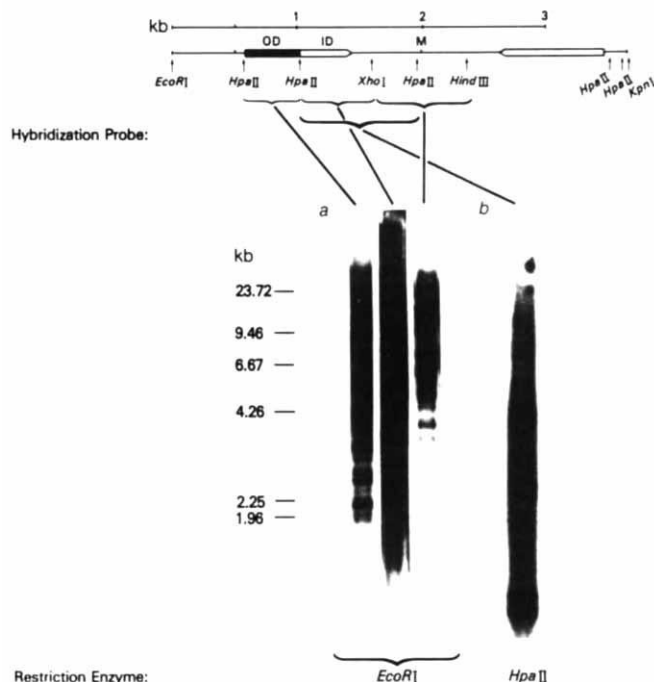


Fig. 6 Southern transfer experiments showing multiple and heterogeneous copies of TU1 sequences in the sea urchin genome. *a*, Autoradiographs of Southern blots of *S. purpuratus* genomic DNA digested with *EcoRI*, which does not cut within TU1, and hybridized to the TU1 outer domain (OD), inner domain (ID) and middle (M) regions of TU1 as indicated. *b*, Autoradiograph of a Southern blot of *S. purpuratus* genomic DNA digested with *HpaII* which cuts internally in TU1 (see figure), and hybridized to the internal *HpaII* fragment indicated.

Methods: In both *a* and *b*, *S. purpuratus* DNA from a single individual was digested with the appropriate restriction enzyme (see figure), electrophoresed on a 0.65% (*a*) or 1.5% (*b*) agarose gel (1.5 µg DNA per well). The gel was blotted onto nitrocellulose as described by Southern³⁷. The blots were preincubated in hybridization buffer (4×SSC, 50 mM sodium phosphate pH 7, 0.05% each of BSA, Ficoll and polyvinylpyrrolidone) for 3–6 h at 65°C and then incubated at the same temperature in hybridization buffer containing the appropriate nick-translated probe (see figure) at ~0.5 × 10⁶ c.p.m. ml⁻¹. Blots were washed in 0.1×SSC, 0.1% SDS, 50 mM sodium phosphate pH 7 at 48–50°C for 3 h and exposed to prefogged X-ray film (XR5, Kodak) at -80°C for 12 h. Shorter exposure times produced autoradiographs with no greater resolution of bands above the intense smears, which represent highly heterogeneous populations of TU1-homologous sequences in the genome.

We assume that the H2B sequences in which TU1 was found is a pseudogene and thus susceptible to random nucleotide mutation without having an effect on function. Because only limited differences are seen between the H2B pseudogene sequence and the functional H2B early gene sequence, formation of this pseudogene as well as the subsequent insertion of TU1 must have occurred relatively recently in the history of the genetic line represented by the individual in whose genome TU1 was discovered.

At least two different mechanisms are believed to be involved in transposition events. In bacteria, transposition occurs by mechanisms that involve duplication of the transposon DNA². It has been suggested that transposition of certain eukaryotic mobile elements such as the *copia*-like elements in *Drosophila*⁴, the retroviral proviruses⁵ and the dispersed repetitive *Alu* sequences in humans³¹, move by a mechanism that involves reverse transcription of mRNA. However, the structure of TU1, as well as the foldback elements of *Drosophila*, is similar to the

structure of bacterial transposons, raising the possibility that this distinct class of eukaryotic transposable elements might transpose by a mechanism similar to the transposition process occurring in bacteria.

The TU1 element family and TU1 itself have some similarities to the transposons formed by insertion of duplicate copies of bacterial insertion sequence (IS) elements. In bacteria, segments consisting of both the IS elements themselves and the segment between them can undergo transposition²; it remains to be determined whether the IVRs of TU1 are themselves transposable elements. Studies now underway involving structural

characterization of additional members of the TU1 family, analysis of their occurrence and genomic organization in different sea urchin species, and analysis of TU1-like elements in other animal species may help to elucidate the possible evolutionary and regulatory roles of TU1 elements and the mechanisms involved in their transposition.

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LETTERS TO NATURE

Mass composition of high-energy cosmic rays and scaling violation in their interactions

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There has long been controversy over the question of mass composition and interaction characteristics of cosmic rays with energies above $\sim 10^{12}$ eV since in this region direct determination of particle mass has not been possible and accelerator data on interaction mechanisms have been scarce. Accelerator results in the 1970s led to the suggestion that particle interactions could be represented by the so-called scaling of the type enunciated by Feynman¹. In this model² an asymptotic condition is reached by 10^{11} eV such that most interaction energy is carried off by a small, constant number of secondaries. Studies of cosmic rays showed that by 10^{15} eV if scaling remained valid then the primary mass composition must have changed considerably. An alternative model³ assumes that the mass composition remains unchanged, in which case the interaction characteristics would have to be very different⁴. The advent of the pp collider results, corresponding to an energy (with respect to a stationary target) of $\sim 2 \times 10^{14}$ eV, now allows us to make a distinction between the two models. Here we present evidence supporting the view that the mass composition of high-energy cosmic rays is not much different from that of lower energy rays, but the scaling model is invalid.

The development of accelerators has led to a reduction of cosmic ray investigations, with most studies restricted to energies above those available in accelerators. At these high energies not only is there the problem of low particle rates but

also the uncertainty in the mass composition of the primaries. The apparent applicability of scaling to p-p interactions at 10^{11} eV (ref. 5) and the claim that the asymptotic condition had been reached making scaling valid at all higher energies, coupled with the observation of an energy spectrum of iron nuclei flatter than that of protons⁶, led to suggestions that iron should predominate by 10^{14} eV. Other arguments have also been put forward⁷ claiming that protons are negligible at 10^{15} eV.

Our case for the contrary view has, until recently, been indirect, the main arguments being the non-applicability of linear extrapolation of the observed power law spectrum of iron³, the presence of fluctuations in shower development at $E_p \sim 10^{15}$ eV (indicating many protons) and other characteristics of EAS, particularly those detected at mountain altitudes. Such arguments have been strengthened recently by independent analysis of the non-validity of extrapolation⁸, and new more precise EAS fluctuation studies⁹ which give no support for an increase in the fraction of iron nuclei ($\approx 15\%$) in the energy range 10^{15} - 10^{17} eV. In addition, preliminary results from the JACEE experiment¹⁰, involving a complex balloon-borne detector, have indicated no iron enhancement to at least 5×10^{13} eV.

Support for the view that strict scaling had not in fact been reached by 10^{11} eV came from our detailed analysis of the ISR results at 10^{12} eV (ref. 11), in which we introduced the concept of a scaling-breaking parameter applied to the usual scaling formulation to give

$$\frac{1}{\sigma_t} \frac{d\sigma}{dp_1 dp_t} = \frac{1}{E_\pi} \left(\frac{s}{s_0} \right)^\alpha \cdot f \left(\frac{p_1}{p_{\max}} \cdot \left(\frac{s}{s_0} \right)^\alpha, p_t \right)$$

where σ_t is the total cross-section for a p-p collision in which a secondary pion has momentum components p_1 (longitudinal) and p_t (transverse), and energy E_π . The parameter α represents the degree of scaling-violation between the C-system energy $\sqrt{s_0}$ and energy $\sqrt{s}$; $\alpha = 0$ would correspond to strict scaling; we made the case for $\alpha = 0.13$ to 10^{12} eV (ref. 11).

The new pp data^{12,13} at $E_0 \approx 2 \times 10^{14}$ eV ($\sqrt{s} = 540$ GeV) indicate that scaling violation continues, and indeed worsens.

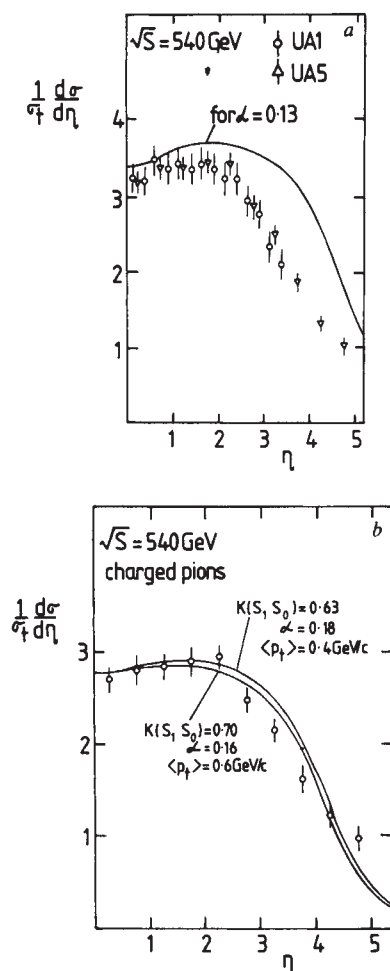


Fig. 1 *a*, The observed and predicted pseudorapidity distributions for all charged particles produced in $p\bar{p}$ collisions at $\sqrt{s} = 540$ GeV. The experimental data are from the work of Arnison *et al.*²¹ and Alpgard *et al.*²². The prediction is for $\alpha = 0.13$; such scaling-breaking is inadequate. *b*, As for *a* but for charged pions alone and with predictions for the parameters indicated.

Figure 1 shows the situation. There is a complication in that the mean transverse momentum is changing and also the inelasticity, with the consequence that a unique prescription is not possible. However, the trend towards increased scaling breaking seems undeniable. Taking 205 GeV as a datum¹², we can write the energy spectrum of pions as

$$P(E_\pi) = (E_0/205)^\alpha q((E_\pi/E_0)(E_0/205)^\alpha) k(s, s_0)/E_\pi$$

where the inelasticity parameter $k(s, s_0) = K_\pi(s)/K_\pi(s_0)$. Choices of the parameters which fit are indicated in Fig. 1. We regard a reasonable choice as

$$\alpha = 0.815((E_0/205)^{0.135} - 1)/(\ln(E_0/205))$$

for $E_0 < 7.7 \times 10^4$ GeV,

$$\alpha = 0.072 + 0.0163 \ln(E_0/205)$$

for $E_0 > 7.725 \times 10^4$ GeV, $K_{\pi(\text{tot})}(s, s_0) = (s/s_0)^{-0.042}$ and $K_\pi \pm (s, s_0) = (s/s_0)^{-0.061}$.

The above expressions clearly have validity to $\sqrt{s} = 540$ GeV but extrapolation to higher energies becomes increasingly problematical.

The question of mass composition of cosmic rays above 10^{12} eV can now be examined by using our model to predict various cosmic ray properties. Probably the most accurate and relevant is the number of muons expected in EAS as a function of shower size, at sea level^{13,14} and at mountain altitudes¹⁵, and calculations have been made for these situations. The relations

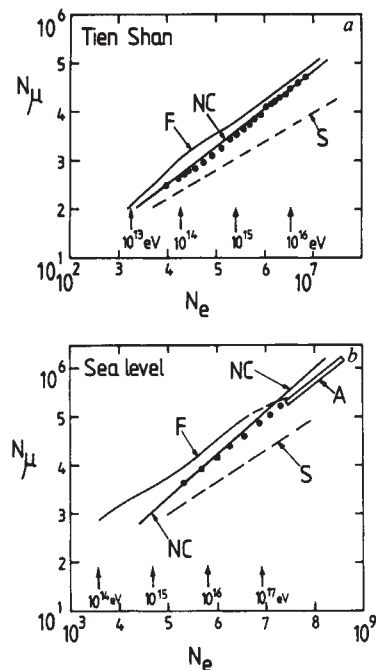


Fig. 2 *a*, Mean number of muons above 5 GeV, N_μ , versus electron number, N_e , for showers at Tien Shan (700 g cm^{-2} depth). The experimental points are from Danilova *et al.*¹⁵. The lines refer to our predictions for scaling-breaking with variable α and normal mass composition (NC) or dominant iron (F). S denotes strict scaling above 2×10^{11} eV. Approximate primary energies are indicated. *b*, Mean number of muons above 10 GeV near sea level versus electron number, N_e . The experimental points are from the Moscow¹³ and Akeno¹⁶ experiments (the latter, denoted A, are normalized to the Moscow value at 10^7 particles).

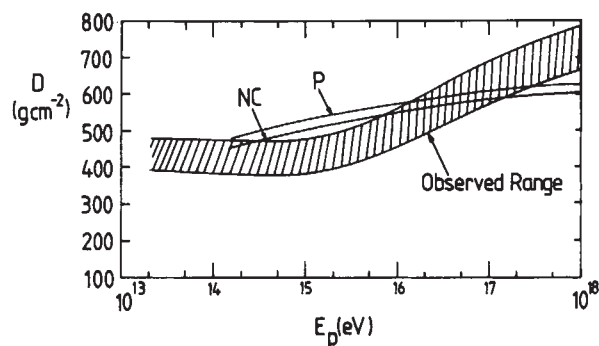


Fig. 3 Depth of shower maximum versus primary energy from a summary described elsewhere²⁰. NC and P denote 'normal composition' and 'protons', respectively. The predictions are for scale-breaking with variable α to the extent described in the text.

just described have been used, together with a rising p - p cross-section (specifically $\lambda = 90(E_0/100)^{-0.05} \text{ g cm}^{-2}$ for protons). Kaons and baryons have been included, to the extent indicated by the accelerator results. An important assumption (to be returned to below) is that the 'superposition model' is valid, that is, that a nucleus of mass A behaves as the superposition of A independent nucleons insofar as the production of the important secondary meson component is concerned. Strong support is available for this model at least as far as 20 GeV per nucleon¹⁶ ($\sim 10^{12}$ eV for an iron nucleus).

Figure 2 shows the results for N_μ versus N_e . Two variants of the mass composition were taken: (1) the 'normal' composition (NC), adopting power laws for the various components all with the same exponent at production (the different observed exponents at energies below about 10^{12} eV being due to fragmentation losses³) and (2) the 'iron-enhanced' composition (F), where iron dominates by 10^{14} eV but the composition returns

to 'normal' by 10^{17} eV (this condition is accepted to be a requirement because of the demonstration of fluctuations in shower development at sea level at such energies¹⁷).

It is important to stress that whereas the predictions for NC represent an extrapolation from accelerator experiments, using our specification, above 2×10^{14} eV, those for F represent firm predictions up to 1.1×10^{16} eV ($56 \times 2 \times 10^{14}$ eV). Inspection of Fig. 2 shows that there is strong evidence against the F composition from both the sea level and mountain results. Over most of the energy range, NC is seen to fit the data although at the highest energies, $E_0 \approx 10^{17}$ eV, there is a deficit of energetic muons at sea level and, if the interaction model is correct at these energies, a composition more nearly purely protonic gives a better fit. Such a composition would not be inconsistent with the EAS data.

It might be thought that the case for ion dominance could be redeemed by invoking an exotic form of interaction for these particles, in which the superposition model is not valid for energies above 10^{14} eV. It is true that there is the possibility of the production of 'quark matter' in nucleus-nucleus collisions¹⁸ and support for the idea has come from the observation of a very high multiplicity ($\approx 10^3$ charged secondaries) in the collision of a silicon nucleus of $\approx 10^{14}$ eV with a 'silver bromide' nucleus. Furthermore, there is a little evidence from α - α collisions (ISR) at 60 GeV (ref. 19) favouring the production of high temperature matter.

It is our view that the case against significant quark-matter effects being important in iron-air nucleus interactions at 10^{14} eV is strong when consideration is given to the geometry of the collisions. We would expect the effects to be strongest in central collisions which, although comprising $\approx 45\%$ of Fe-Ag interactions, are only responsible for about 10% of Fe-air nucleus collisions. The likelihood of generating quark matter in the common peripheral collisions seems to us to be quite negligible.

Finally, important results have been obtained on the dependence of the depth of shower maximum on primary energy. This dependence was much used in earlier work (for example, ref. 4) to claim a breakdown of scaling. Figure 3 summarizes the present situation, and reveals agreement at energies below about 2×10^{14} eV but a poor fit between 3×10^{14} eV and 10^{16} eV. Primary iron nuclei cannot be pressed into service for the reasons given above, and it must be concluded that, unless the measurements are wrong, there is evidence for an even stronger scaling breakdown in this range, at least in the constituent part leading to the predominantly electronic component which determines the depth of maximum. Above 10^{17} eV there is again disagreement between theory and experiment. A possible explanation is that the scaling-breaking parameter, α , saturates at 10^{16} eV or so but many other changes, perhaps associated with kaon and baryon production, are also possible.

Thus the scaling-breaking observed at 10^{12} eV is even greater at 10^{14} eV and appears to continue to 10^{16} eV; indeed a simple extrapolation to 10^{15} eV may not give sufficiently high secondary particle multiplicities to enable an explanation of the depth of maximum of EAS. The energy range 3×10^{14} eV to 10^{16} eV may be unique in this respect and less severe scaling-breaking appears adequate above 10^{17} eV.

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Low-frequency polarimetry of the millisecond pulsar PSR1937+214

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Pulsars with periods of a few milliseconds^{1,2} test theories of the radio emission process in extreme conditions. The velocity of light radius, which is an important scale-length in most theories, is less than 10 stellar radii for the millisecond pulsar PSR1937+214, and the inferred surface magnetic field is a few times 10^8 G, less than 10^{-3} that inferred for the Crab pulsar. Polarization observations across both the main pulse and the prominent interpulse can help clarify the emission geometry and probe similarities between this pulsar and others, such as the Crab pulsar, that have very different magnetospheric conditions. High-frequency polarization observations exist for this pulsar^{3,4}, but the temporal smearing produced by interstellar dispersion has hampered low-frequency observations. We now report polarization observations at 431 MHz that have 4 μ s time resolution and are free from the effects of interstellar dispersion. We find that the interpulse polarization decreases towards lower frequencies, opposite in trend to the polarization of the main pulse and of most other pulsars. The position angles at the centre of the main pulse and interpulse are approximately equal and the position angle gradients have the same sign, supporting a model in which the main pulse and interpulse are emitted above opposite magnetic poles.

The observations were made on 3 April 1983 using the 305-m telescope at the Arecibo Observatory. Orthogonal circular polarizations were passed through 250-kHz Butterworth filters at a centre frequency of 431 MHz. Phase quadrature baseband mixers separated the signal into real and imaginary components that were sampled at the Nyquist interval of 4 μ s.

Since this procedure preserves the phase information in the signal, the effect of interstellar dispersion can be removed⁵. The details of this technique are discussed elsewhere⁶. Briefly, processing consists of convolving the complex time series with a filter whose phase values are the negative of those introduced by the interstellar medium. For speed, this convolution is done as a multiplication in the frequency domain by an array processor. Transformed back to the time domain, the complex samples in the L and R polarizations are multiplied in the combinations LL*, LR*, L*R and RR* to form power quantities that are averaged and converted into Stokes parameters. Calibration of the polarization data was accomplished by observing the unpolarized continuum source 3C357, which was assumed to have a flux density of 7.0 Jy at 431 MHz.

A synchronous average of 8.2×10^5 pulses is shown in Fig. 1, smoothed to 16 μ s to improve the signal-to-noise ratio. The stronger of the two pulses corresponds to the high-frequency main pulse, since multi-frequency timing observations⁶ preclude

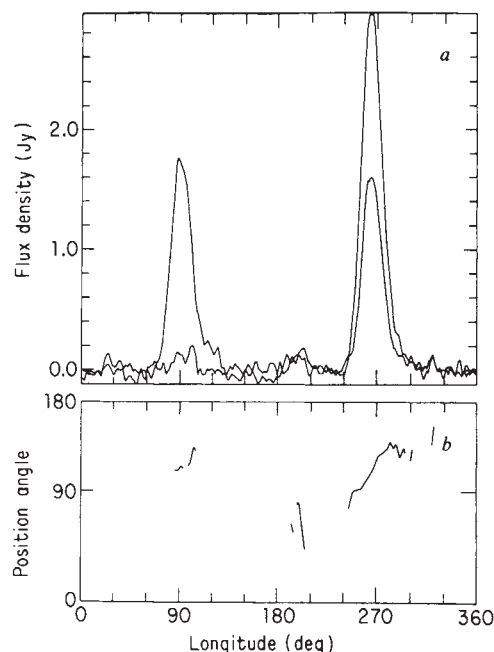


Fig. 1 Polarization waveform of PSR1937+214 at 431 MHz. This is a synchronous average of 8.2×10^5 pulses, smoothed to a time resolution of $16 \mu\text{s}$ or 3.6° of longitude. *a*, Plot of the intensity (outer envelope) and linear polarization. The circular polarization is consistent with zero across the entire pulse period and is omitted for clarity. The position angle, shown in *b*, is only plotted when the linear polarization is greater than twice the off-pulse (zero-centred) standard deviation. This limits errors in the position angle to approximately $\pm 15^\circ$, with much smaller errors across most of the main pulse. The flux density scale is only accurate to $\pm 30\%$ due to pulse strength variability, and the origin of the position angle scale is arbitrary.

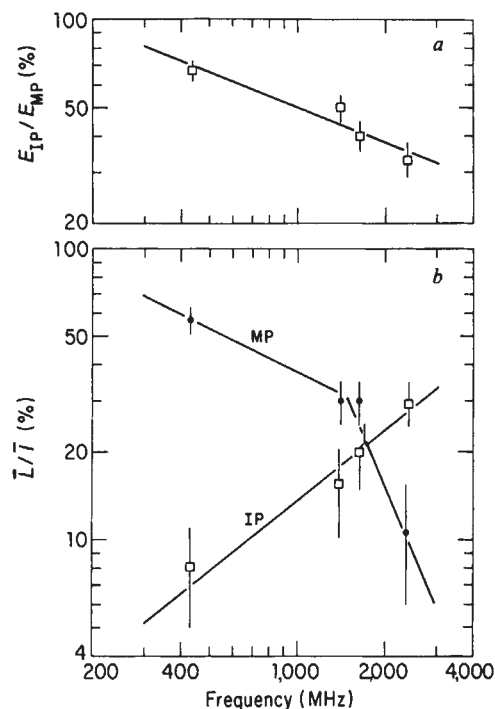


Fig. 2 *a*, The ratio of integrated interpulse (E_{IP}) to main pulse (E_{MP}) energies shown as a function of frequency. A power-law curve has been fitted by eye. The high-frequency data here and in *b* are taken from refs 3 and 4. *b*, The ratio of integrated linear intensity (L) to total intensity (I) shown for the interpulse and main pulse as a function of frequency. Approximate power-law dependencies have been fitted by eye. The main pulse behaviour is similar to that seen in many pulsars whereas the interpulse behaviour is anomalous.

the possibility that the main pulse and the interpulse have switched identities between 431 and 1,415 MHz. The ratio of interpulse to main pulse integrated intensity is plotted in Fig. 2*a*, which shows that the pulse energies tend to equalize at lower frequencies. There is a suggestion of a third pulse component that precedes the main pulse by 65° of longitude, perhaps the analogue of the Crab pulsar 'precursor'⁷, however, higher sensitivity observations are needed to explore the relationship of this pulse component to the main pulse.

The decrease in the average linear polarization of the interpulse (as frequency decreases), which had been suggested by higher frequency observations, is confirmed here. Figure 2*b* summarizes the frequency development of the linear polarization of the main pulse and the interpulse. For comparison, the polarization of the Crab pulsar main pulse is $\approx 20\%$ at 410 MHz and $\approx 25\%$ at 1,664 MHz (ref. 8). The average linear polarization of most pulsars is roughly constant up to a critical frequency ($f_{crit} \sim 1,000$ MHz), above which the polarization decreases approximately as f^α , with $\alpha \approx -0.7$ (refs 9, 10). The anomalous interpulse depolarization may indicate an unpolarized emission process or it may result from the equalization of two orthogonally polarized radiation modes at lower frequencies; average polarization data cannot distinguish between the two.

The position angles at the centre of the main pulse and the interpulse are equal to within $\pm 10^\circ$, as compared with the almost orthogonal values observed at higher frequency. As we have not measured absolute position angles at either high or low frequencies, we cannot determine directly whether the main pulse or the interpulse has undergone an orthogonal mode transition. The depolarization of the trailing edge of the interpulse at 1,415 MHz, however, and the low value of interpulse polarization at 431 MHz make it likely that the interpulse has changed its dominant polarization state. The positive position angle gradient across both pulses agrees with the 1,415 MHz

result and with the 2,380 MHz data if the sign convention of the 2,380 MHz data is incorrect⁴.

Although the data at neither high nor low frequency are yet of adequate sensitivity and time resolution to be conclusive, the observations favour a model in which the main pulse and interpulse arise above opposite magnetic poles (see refs 11, 12 for a discussion of one-pole and two-pole interpulse models). The near equality of the main pulse and interpulse position angles and the same sign of position angle gradient across the pulses support a model in which the star's magnetic axis lies close to the rotational equator (perpendicular rotator). In order to account for identical signs of the position angle gradients, the angle between the line of sight to the star and the rotational equator must be larger than that between the magnetic axis and the rotational equator¹².

Recently, Narayan and Vivekanand¹³ have suggested that pulsar beams are elongated (with the latitudinal axis about three times the longitudinal axis) and that this elongation is more pronounced in shorter-period pulsars, providing a simple explanation for the much higher incidence of interulses among short-period pulsars. The polarization observations presented here are consistent with this model if the line of sight is further from the rotational equator than is the magnetic axis.

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Wind tunnel generation of horizontal roll vortices over a differentially heated surface

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Wildland crown fires represent an extreme type of forest fire that advances from top to top of trees or shrubs. Some such fires generate energy comparable to several nuclear explosions. They occur most frequently in coniferous forests found in the United States and Canada, and in broad-leaved eucalyptus forests of Australia. Here we report visualization of wind tunnel experiments in which airflow along the perimeter of a simulated forest fire produced horizontal vortex pairs with axes in the downstream direction. These vortex pairs formed above a differentially heated surface simulating the thermal energy flux from a crown fire. The thermal energy was produced by an electrically heated surface ribbon at a temperature of $\sim 250^\circ\text{C}$ and also, in a separate experiment, by an electrically heated embedded wire coil at a temperature of $\sim 825^\circ\text{C}$. Wind velocities ranged from 0.2 to 3.6 m s^{-1} . Similar vortex action may cause the unburned tree-crown strips that are observed when crown fires, driven by a significant wind, burn over flat terrane.

Crown fires leave distinctive patterns of burned and unburned vegetation that document the fire's behaviour. For example, long strips (streets) of coniferous crowns with unburned (although usually scorched) needles are often seen in otherwise blackened areas (Fig. 1). In examining unburned crown streets following one New Jersey and two Michigan fires, we found high scorch on the outer (opposite) sides of closely spaced tree trunks with little or no scorch on the inner (facing) sides,



Fig. 1 Unburned tree-crown streets resulting from a 1980 wildland crown fire in Michigan. The dark areas are burned jack pine; white is unburned. The ambient wind direction and the forward fire spread were from right to left.

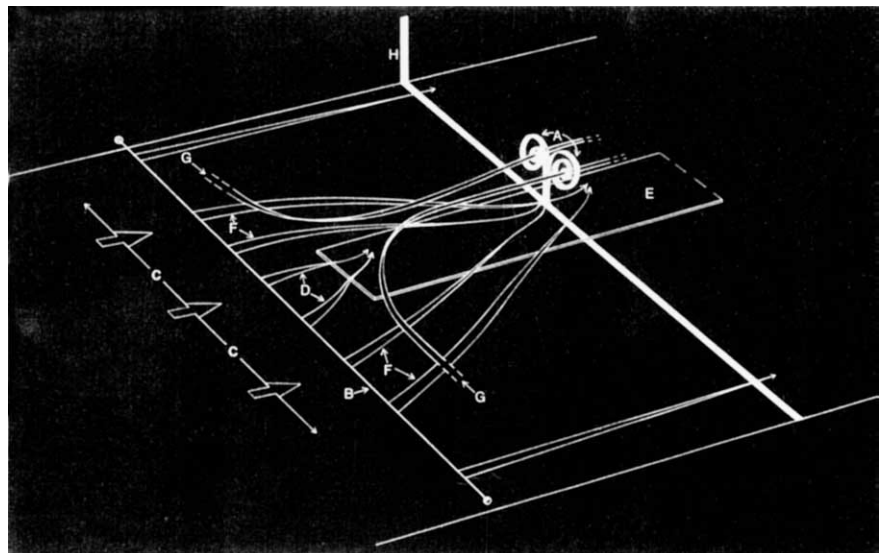
indicating significant airflow outward from within all streets¹. Haines² hypothesized that downward air movement caused by horizontal roll vortices formed these tree-crown streets and trunk-scorch patterns. He suggested that relatively cool air flowing downward from these vortices kept fire out of the crowns, and that as this air neared the ground, it spread out horizontally, in opposite directions from within the street.

A typical free-burning crown fire spreads unevenly; the forward fire perimeter, usually called the 'head', moves most rapidly. The adjoining portions or 'flanks', at right angles to the head, spread laterally. The result is that the flame front, where the major portion of the energy of combustion is released, is concentrated along a dynamic perimeter which assumes the shape of an ellipse that continually enlarges, particularly longitudinally.

The hypothesis of the formation of tree-crown streets² suggests that the action of a single vortex along the perimeter results in a single crown street. As the perimeter enlarges, that vortex dissipates and another vortex forms along the new perimeter, causing a second crown street. Continued formation and dissipation of vortices along the enlarging perimeter would result in a number of crown streets (Fig. 1).

This report describes wind tunnel experiments to test the hypothesis that horizontal roll vortices occur along the flanks of wildland crown fires. Using a wind tunnel with test-section dimensions $23 \times 76\text{ cm}$ (height and width) and 76 cm long, we

Fig. 2 A schematic of the possible airflow pattern during generation of a vortex pair (A) over a heated metal ribbon. A hot wire, coated with light oil to generate smoke, (B) was oriented normal to the airflow, 0.5 cm above the tunnel floor (C). Buoyant forces created an upflow (D) as the air passed over the $2.5 \times 50\text{ cm}$ heated ribbon (E). The streamlines to the sides of the heated ribbon depict the inflow (F) required by conservation of mass. The transverse vorticity of the boundary layer (G) changed to longitudinal vorticity and increased. A vertical opening in the tunnel side (H), located 15 cm downstream from the ribbon's leading edge, allowed sunlight (reflected into the tunnel from two front-surface mirrors) to illuminate a thin cross-section of the smoke-filled airflow.



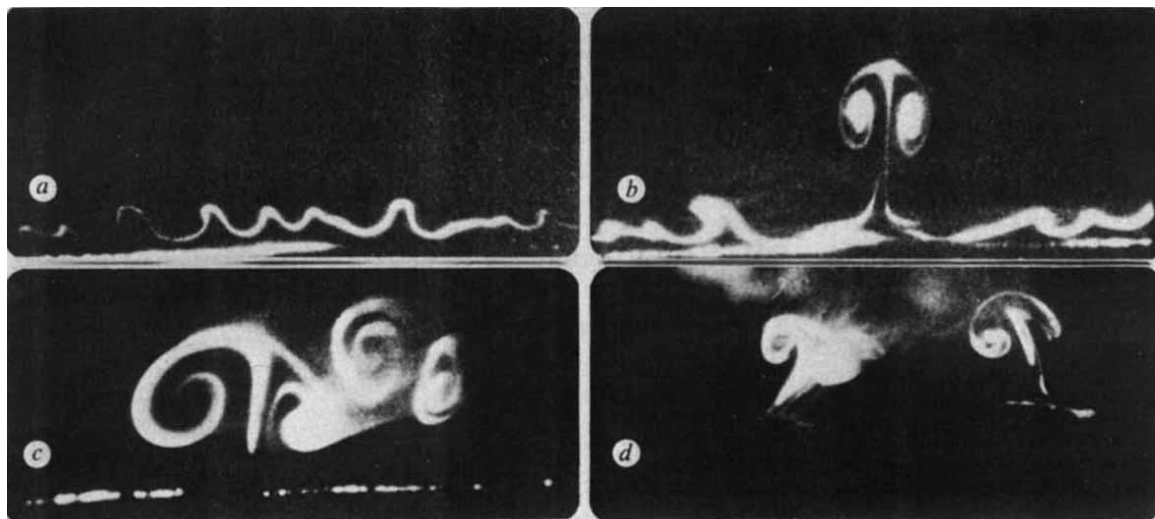


Fig. 3 *a*, A cross-section of the sheet of smoke released from the hot wire (no ribbon heating); *b*, the same cross-section with air flowing over the heated ribbon producing buoyancy and a vortex pair; *c*, eddying occasionally caused distortion of one vortex, and it changed into a detached, secondary vortex pair; as the airflow steadied, these transient forms stabilized, resuming the structure seen in *b*. *d*, Two vortex pairs generated along both flanks of a simulated elliptical perimeter. The embedded heated coil is partially visible as a line at the base of the plume.

conducted two experiments simulating the airflow along a fire perimeter. The configuration in Fig. 2 shows the first experiment simulating one flank of a crown fire; in the second experiment an elliptical coil of heated wire simulated the head and both flanks. Airflow speeds varied from 0.2 to 3.6 m s⁻¹. Photographs were taken with an Arriflex 16S/B camera positioned downstream at the tunnel exit at about a 20° angle off the axis of the flow.

We began the experiments by observing first the effects of heating over a small, flat plate without forced convection. The results proved uneventful in that we did not observe roll vortices. These preliminary observations with a spot source of thermal energy suggested that free convection roll vortices do not occur as a steady-state phenomenon without forced convection. Of course, free convection vortex structures in enclosed containers do occur and have been well documented, see for example, Bénard cells.

The illuminated cross-section of airflow without ribbon heating showed a relatively stable smoke pattern stretching across the width of the tunnel floor (Fig. 3*a*). With the ribbon heated to 250 °C, a thin, buoyant plume developed along the length of the ribbon, its height inversely related to the airflow velocity (Fig. 3*b*). A vortex pair developed at the top of the plume over the full range of air speeds. The vortex pair height ranged from one half to three times the ribbon width.

Although the tunnel airflow was essentially stable, eddying caused momentary changes in the structure of the plume and vortex pair (Fig. 3*c*). The smoke flow indicated prevailing streamlines resembling the pattern shown in Fig. 2. It appears that an underlying cause for the vortex pair is the reorientation of boundary-layer vorticity from a transverse axis to a longitudinal axis. This phenomenon has been observed in other laboratory situations and in at least one instance in a large-scale controlled burn conducted during a light wind³.

A second experiment, involving an elliptical coil heated to 825 °C, simulated a complete perimeter. The shape of a wind-driven crown fire was approximated by assuming an ambient windspeed of 7 m s⁻¹ and joining two ellipses with a crosswind dimension of 17.5 cm and a streamwise dimension of 37.5 cm (ref. 4). At moderate flow velocities (1.2 m s⁻¹), the shapes of the vortex pairs in this second experiment appeared as in Fig. 3*d*. Increased airflow speed reduced the height of the plume and vortex pairs. The highest speeds resulted in vortex pairs forming very close to the tunnel floor with apparently increased vorticity. Although the base of the plume remained stable at higher speeds, the plume and vortex pair tended to weave slowly back and forth.

These experiments differ from previous laboratory studies in that a differentially heated surface was used to influence the airflow. Most research of this nature has been concerned with heat transfer, without regard for the flow patterns induced by differential buoyant forces (see for example, ref. 5). However, in studies emphasizing flow between two parallel plates held at different temperatures (see for example, ref. 6), longitudinal (downstream) vortices appeared when the flow was inclined to the horizontal. Such vortex structures exist in isothermal flows and are not directly related to the roll vortices generated with differential heating in the present experiments.

The downstream vortex pairs described here resemble those observed during an experimental burn involving a 140 × 140 m square filled with fuel oil burners³. In that study a large vortex configuration was associated with the buoyant plume during moderate surface winds (4–8 m s⁻¹). The smoke column above the burners bifurcated into two counter-rotating vortices. As they rose and approached neutral buoyancy and environmental windspeed, the vortex pair became horizontal.

There is always a question of scaling when using small models such as these to represent large-scale natural phenomena. Modelling studies of large fires^{7,8} indicate that one can be reasonably confident of results if one does not violate basic scaling principles. As these first experiments duplicated a fluid dynamic process over flat terrain using geometrically-simple fire models, major scaling complexity was eliminated, suggesting that full-scale interpretation should be possible.

These initial results support the tested hypothesis and suggest that downstream vortices may be a common boundary layer structure in wildfires where burning is concentrated along the fire's flanks. Because these vortices generate large-scale secondary flows capable of transporting firebrands, they may be a major factor in lateral fire spread. Lateral spreading is an important consideration in firefighter safety.

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Capillary Mössbauer spectroscopy for solution chemistry

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Mössbauer spectroscopy is highly sensitive to changes in the electric field gradient and electronic density around atomic nuclei, but cannot be used to study the electronic species of compounds in solutions because the necessary recoilless γ -ray resonance absorption has so far only been achieved in solids. We recently suggested¹ the use of a Corning Vycor porous ('thirsty') glass^{2,3} carrier for the Mössbauer study of liquids. Here we show that the Mössbauer effect can be achieved in liquid samples trapped in the pores of this glass carrier, providing a new technique which may be a useful tool in solution chemistry.

Figure 1 shows the room temperature Mössbauer spectrum of an aqueous 0.5 M iron(II) sulphate solution trapped in the 4-nm pores of the glass carrier. The recoilless γ -resonance absorption in such samples has naturally a much lower intensity than in solids. For the spectrum in Fig. 1, which was recorded for 4 days, the isomer shift (1.25 mm s^{-1} , referred to α -ion) and quadrupole splitting (3.17 mm s^{-1}) values were calculated by the conventional computer evaluation method with an adequate accuracy ($\pm 0.01 \text{ mm s}^{-1}$).

It is known from coordination chemistry that an aqueous solution of iron(II) sulphate contains the hexaquo iron(II) species. In crystalline $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ the iron(II) is in the same form. We therefore measured the Mössbauer parameters of aqueous iron(II) sulphate solution at room temperature in the carrier and compared them with those of iron(II) sulphate crystals at the same temperature (Table 1). Isomer shift and quadrupole splitting of iron(II) in the solution were found to be identical (within the experimental error) with those measured in the $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ crystal, but differed from parameters taken with other solid iron(II) sulphates.

This suggests that the Mössbauer parameters of the liquid trapped in the pores of thirsty glass reflect the electronic structure of the species in the original solution. Eventual interaction with the glass surface in the pores of the carrier did not influence the Mössbauer pattern.

Figure 2 shows the Mössbauer spectra of 0.01 M aqueous solutions of the low spin tris(2,2'-dipyridyl)iron(II) complex frozen and measured at 80 K (Fig. 2a), trapped in the pores of 'thirsty' glass and frozen and the spectrum measured at 80 K (Fig. 2b), and trapped in the pores of thirsty glass and measured at room temperature (Fig. 2c). The Mössbauer parameters are presented in Table 2. The complex was prepared from iron enriched in ^{57}Fe .

The Mössbauer parameters measured at 80 K (liquid nitrogen temperature) in the original solution and in that trapped in the pores appear to be identical. The data taken at room temperature showed the expected temperature shift but not their changes, so the effect of the interaction between the glass surface and the Mössbauer-active solute appears negligible in the latter system also.

The subordinate role of this interaction in both systems can be explained on the basis of our recent differential scanning calorimetric (DSC) data which show that bulk water has a higher affinity for the surface in the pores of thirsty glass than solvate water bound by solutes. Thus bulk water covers the internal surface of the carrier separating it from the species in solution.

Table 1 Mössbauer parameters of iron(II) sulphate systems at room temperature

	Isomer shift (mm s^{-1})	Quadrupole splitting (mm s^{-1})
Aqueous solution	1.25	3.17
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	1.25	3.20
$\text{FeSO}_4 \cdot \text{H}_2\text{O}$	1.23	2.70
FeSO_4 anhydrous	1.31	2.94

Table 2 Mössbauer parameters of tris(2,2'-dipyridyl)iron(II) in aqueous solutions

	Temperature (K)	Isomer shift (mm s^{-1})	Quadrupole splitting (mm s^{-1})	Integral intensity
In carrier	293	0.30	0.30	0.002
In carrier	80	0.35	0.33	0.021
Original solution	80	0.35	0.34	0.022

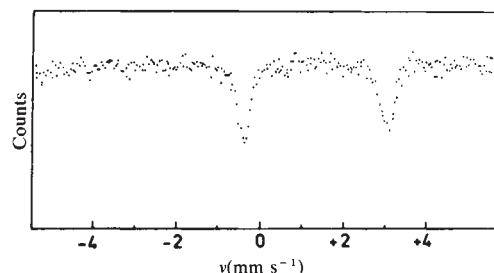


Fig. 1 The Mössbauer spectrum of 0.5 M aqueous iron(II) sulphate solution trapped in the pores of the thirsty glass carrier, measured at room temperature.

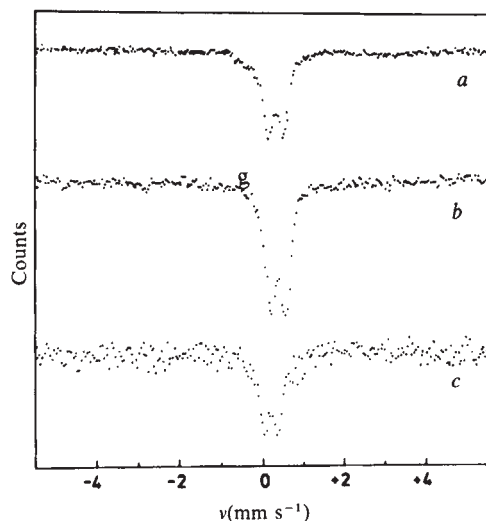


Fig. 2 The Mössbauer spectra of the 0.01 M aqueous solutions of tris(2,2'-dipyridyl)iron(II): a, frozen and measured at 80 K; b, trapped in the pores of thirsty glass, measured at 80 K; c, trapped in the pores of thirsty glass, measured at room temperature.

In order to establish that the Mössbauer spectra arise from the liquids and not from the precipitate of solid crystals in the pores of the glass carrier or from the adsorption of a solid layer on the internal surface of the capillaries, the behaviour of model systems in which both solute and solvent are liquids was studied.

Figure 3 shows the results of a typical example of such a study. The Mössbauer spectrum of the mixture of tetramethyltin and chloroform trapped in the pores of the thirsty glass carrier

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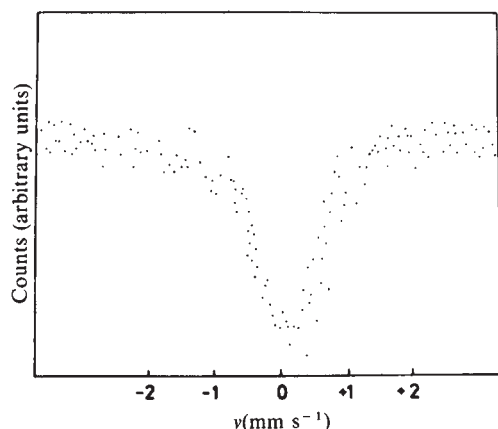


Fig. 3 The Mössbauer ^{119}Sn spectrum of a 1:2 v/v mixture of tetramethyltin and chloroform trapped in the pores of thirsty glass and measured at room temperature. The Mössbauer isomer shift ($\delta = +0.10 \text{ mm s}^{-1}$ referred to SnO_2) indicates the solvation of $(\text{CH}_3)_4\text{Sn}$ by chloroform. This phenomenon will be discussed in detail elsewhere.

was recorded at room temperature, where both components and the mixture are liquids. This experiment proves that the Mössbauer effect can indeed be demonstrated in systems containing only liquid components. Similar investigations of systems containing only liquid components (iron pentacarbonyl in methanol, trimethyl stannic chloride in chloroform and stannic tetrachloride) also gave rise to well evaluable Mössbauer patterns.

In model solutions of low polarity the Mössbauer parameters reflected interactions between the solute and the glass surface⁴, which can be a limitation in the application of capillary Mössbauer spectroscopy in non-aqueous systems.

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A new layered mineral from the Mighei carbonaceous chondrite

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An unusual material that has been called the 'poorly characterized phase' (PCP)¹ is abundant in CM2 carbonaceous chondrites. Its identity has been one of the more difficult problems of carbonaceous chondrite mineralogy. Its puzzling compositions, petrographic textures and X-ray diffraction patterns do not fit any known mineral^{1,2}. The high-resolution transmission electron microscopy (HRTEM) described here reveals an Fe–Ni–S–O layered mineral occurring as a major component of PCP in the Mighei CM2 carbonaceous chondrite. The new mineral exhibits extremely unusual structures, with curved, rolled and wavy layers resembling those of serpentine minerals. It also occurs in a coherent intergrowth with phyllosilicates in various ordered and disordered sequences. The intergrowth is the most common form of the Fe–Ni–S–O mineral and is prevalent throughout the matrix; therefore, this new mineral should be considered an important phase that is genetically related to phyllosilicates in the CM2 carbonaceous chondrites.

Table 1 Electron microprobe analyses (oxide weight percentages except sulphur) of PCP in olivine chondrules and in the matrix in Mighei

	PCP in olivine chondrules*	PCP in the matrix†
FeO	53.6 (49.0–56.5)	55.8 (50.8–59.1)
NiO	7.3 (5.2–10.1)	2.2 (1.7–4.3)
Cr ₂ O ₃	2.5 (1.8–3.4)	<0.1 (0.0–0.3)
P ₂ O ₅	3.1 (1.7–4.9)	<0.1 (0.0–0.4)
S	18.3 (15.7–22.0)	13.2 (5.2–17.1)
SiO ₂	3.0 (1.1–5.9)	8.1 (3.8–13.3)
MgO	4.1 (3.1–5.5)	7.1 (5.7–9.2)
Al ₂ O ₃	0.6 (0.3–0.9)	1.8 (1.5–2.0)
CaO	<0.1 (0.0–0.4)	0.1 (0.0–0.5)
K ₂ O	<0.1 (0.0–0.1)	<0.1 (0.0–0.1)
Total	92.5	88.4

The values in the parentheses show the ranges of the analyses. Although Bunch and Chang³ reported that carbon is contained in PCP up to ~8 wt%, it is not included in our electron microprobe data.

* Mean of 17 analyses from PCP blebs in four different chondrules.

† Mean of 14 analyses from PCP in the matrix.

PCP occurs as isolated grains and clusters of fibrous grains in the matrix and also as spherical or oval blebs in chondrules and aggregates. It contains Fe, S and O as major components and Ni, Cr, P, Si, Mg, Al, Ca, and possibly C in lesser, variable amounts (Table 1). Fuchs *et al.*¹ described it as soft, fibrous, highly magnetic, water insoluble and, for some types, strongly anisotropic in reflected light. It may be amorphous to poorly crystalline, but the fibrous PCP reveals a strong X-ray diffraction peak at 5.4 Å and a weaker peak at 2.72 Å (ref. 1). Bunch and Chang³ observed an inverse correlation between S and Si in PCP and suggested that it must consist of a mixture of an Fe-rich phyllosilicate and an Fe–Ni–S–C phase.

There is independent evidence suggesting that considerable Fe, Ni and S in the matrices must exist in some unknown form; it may be related to PCP. CM2 matrices invariably show S contents from 2 to 4 wt% (ref. 4). However, Fuchs *et al.*¹ found that only a small fraction (0.13 wt%) of the total S (7.24 wt% as FeS) in Murchison can be explained as visible sulphides by direct microscopic observations, and suggested the remainder must be dispersed in the matrix as sub-microscopic grains. McSween and Richardson⁴ observed an apparent correlation between Fe, Ni and S in many CM2 matrices and suggested there must be a phase containing Fe, Ni, S and O that has not yet been identified.

Such a S-bearing phase that is distinct from sulphides would be of special interest because of its potential as a primordial noble-gas carrier. A high concentration of the trapped noble gas in the carbonaceous chondrites was found in an acid-soluble phase that primarily contains Fe, Ni, Cr and S (refs 5–7). However, the identity of the host phase for the noble gases has been highly controversial—is it such an Fe, Ni, Cr and S phase or an acid-insoluble carbonaceous material^{8,9}? The analyses of Bunch and Chang³ show a high concentration of C in PCP. They suggested that the noble-gas-rich carbonaceous material formed before PCP, but was incorporated within it during the alteration process that produced PCP.

Preliminary results from the HRTEM observations of PCP in the Mighei CM2 carbonaceous chondrite have been presented elsewhere^{10–12}.

Specimens of PCP, both in olivine chondrules and in the matrix, were extracted from a petrographic thin section and were ion-thinned for TEM observations. Here we refer to PCP as grains large enough (>1 µm) to be recognized by optical microscopy. Electron microprobe analyses of these specimens are shown in Table 1. Although the compositions of both types of PCP vary widely, they clearly differ from one another. Relative to PCP in chondrules, PCP in the matrix contains higher concentrations of Si, Mg and Al, lower concentrations of S and Ni, and trace amounts of Cr and P.

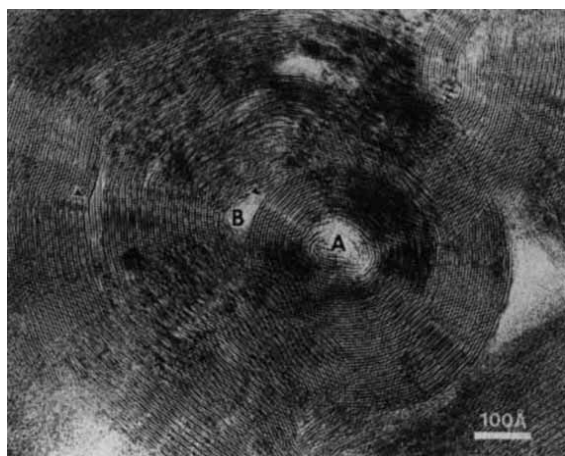


Fig. 1 A high-resolution transmission electron microscopy image of the 5-Å phase from a PCP spherule in an olivine chondrule of Mighei. Terminations and discontinuities of the layers are indicated by arrows. Two distinct core regions are shown by A and B.

HRTEM images from the PCP spherules in chondrules reveal a major constituent that displays complex, fibrous textures typical of a mineral having a layered structure. Layers are commonly curved, rolled and wavy. The interlayer spacing varies considerably but averages ~ 5.4 Å, and in many places it shows a ~ 10.8 -Å periodicity that corresponds to a doubling. The longer periodicity presumably results from an ordered superstructure based on the 5.4 Å layers. We tentatively refer to all of this material as the '5-Å phase'.

Figure 1 shows a HRTEM image of such rolled layers of the 5-Å phase. The image is a cross-section of a cylindrical tube, sectioned perpendicular to the fibre axis. The central tube, with a diameter about 300 Å, shows a well-developed concentric structure. However, the outer layers are concentric only on the right side of the core (A in Fig. 1); on the left side, they separate into an elongated, almost polygonal form that seems, in part, concentric around a distinct core region (B in Fig. 1). Dislocations such as terminated and discontinued layers occur in many places.

Energy-dispersive spectra obtained by TEM from the 5-Å phase reveal a strong Fe peak and less intense S and Ni peaks. No significant amounts of lithophile elements were detected. We used electron energy loss spectroscopy (EELS) to search for light elements; the 5-Å phase displays a strong oxygen peak.

Another significant feature is that small particles (<0.5 µm across) of magnetite, chromite and Fe-phosphate commonly occur in association with the 5-Å phase. They are entangled in the fibres and are also contained as lenticular grains between layers. The minor Cr and P that we invariably detected in PCP spherules in olivine chondrules (Table 1) are presumably contained within these submicrometre particles.

The matrix PCP shows another outstanding feature of the 5-Å phase: it is commonly coherently intergrown with a layered material having an interlayer spacing of ~ 7 Å (Fig. 2). The two kinds of layers show various ordered and disordered sequences that form fairly large, platy crystals (~ 0.1 µm across) typically displaying slight curvatures. The most common ordered sequence has a regular repeat of ~ 17 Å that consists of 7-, 5- and 5-Å layers. [Properly speaking the spacing should be ~ 17.8 Å, but for consistency with previous reports, we use here 17 Å.] Based primarily on the basal spacings and bulk compositional data, such intergrowths were tentatively identified as a new mixed-layer type consisting of serpentine-like and brucite-like materials^{13,14}.

Figure 2a and b shows interfaces between the 7-Å and 5-Å layers and between the 7-Å layers and 17-Å mixed layers. We used energy-dispersive spectroscopy (EDS) on our TEM for characterizing the different layer types. The 7-Å layer region contains major Fe, lesser Si and relatively small amounts of Mg

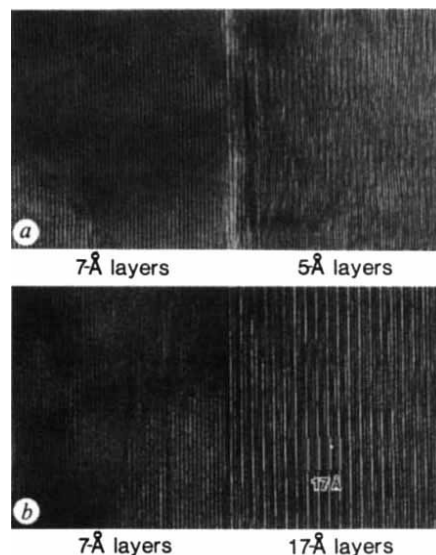


Fig. 2 Interfaces between regions containing: a, the 7-Å and the 5-Å layers, and b, the 7-Å layers and the mixed layers ($\sim 7-5-5-$). Note the textural differences between the 5-Å and 7-Å layers in a: the 5-Å layers are typically rippled and contain many dislocations, while the 7-Å layers exhibit a straight, parallel arrangement.

and Al; its composition and structure suggest it is an Fe-rich, serpentine-type phyllosilicate. The spectrum from the 5-Å layer region shows the same features as the 5-Å phase in the PCP spherules. The mixed-layer region combines the peaks from the above two spectra, indicating that the mixed layers of 5 and 7 Å constitute an intergrowth of the 5-Å phase and the Fe-rich phyllosilicate. The lack of significant Mg indicates that brucite can be eliminated as a possibility for the 5-Å layers.

The TEM observations indicate that PCP contains high concentrations of the 5-Å phase. PCP turns out not to be a single phase, but is actually an intergrowth of at least three phases: (1) the 5-Å phase, (2) the Fe-rich, serpentine-type phyllosilicate, and (3) submicrometre grains of magnetite, chromite and phosphate. These phases are mixed in various proportions, resulting in the wide compositional variations of PCP. Compositional differences observed between PCPs in chondrules and in the matrix (Table 1) are consistent with our TEM results that revealed the former consists mostly of phase (1) and a minor amount of phase (3), and the latter consists mostly of phases (1) and (2).

The 5-Å phase was also found in other CM2 meteorites^{11,12} besides Mighei. It occurs in PCP as well as in areas rich in phyllosilicates, where it occurs as coherent intergrowths on a unit-cell scale. It is clearly an important phase in the CM2 carbonaceous chondrites and is responsible for a large portion of the S content in the matrices. The 5-Å phase is probably the same as the Fe-Ni-S-O phase that McSweeney and Richardson⁴ predicted to exist widely in CM2 matrices and as the PCP sulphide mentioned by Barber¹⁵.

We are unable to correlate the 5-Å phase with any terrestrial mineral. Based on the elements present, it may be an Fe-sulphate. (We have learned that Dr Barber's group described a similar phase from other CM2 meteorites and suggested it may be a hydrous sulphide.) The only minerals known to show similar cylindrical and wavy structures are chrysotile and antigorite¹⁶⁻¹⁸, in which the structural irregularities result from a small dimensional misfit between two different types of joined sheets. However, the interlayer spacing of the 5-Å phase is too small to accommodate two distinct types of layers, and so it is obvious that the 5-Å phase needs another explanation for its unusual structures.

Bunch and Chang³ argued that PCP in the CM2 matrices is a low-temperature aqueous alteration product of anhydrous materials in a parent body regolith. We observed the 5-Å phase

in PCP replacing a large kamacite grain in a chondrule of Mighei¹⁰, which provides clear evidence that at least some 5-Å phase was produced by alteration of kamacite.

The common intergrowth of the 5-Å phase with the Fe-rich phyllosilicate indicates they probably formed simultaneously, presumably in a low-temperature, hydrous, Fe- and S-rich environment. Phyllosilicate formation in a S-rich environment is not uncommon terrestrially. During serpentinization of olivines and pyroxenes in ultramafic rocks, a variety of sulphides form as disseminations in serpentine minerals¹⁹⁻²¹. Nevertheless, no intergrowths of the types we report here are known from such terrestrial samples. It remains to be determined whether this is (1) because such samples have simply not been examined in the ways that we describe here and the phases have thus been overlooked, or (2) because the CM2 meteorites experienced a unique physicochemical environment for developing these unusual materials.

A sample of Mighei (USNM3483) was supplied by R. S. Clarke, from the National Museum of Natural History, Smithsonian Institution. We thank I.D.R. Mackinnon for useful discussions and J. Clark for assistance with electron microprobe analyses. The microprobe was obtained partly with funds provided by the NSF. Electron microscopy was performed at the electron microscope facility in the Center for Solid State Science at Arizona State University. This work was supported by NASA grant NAGW-143.

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An X-ray structural study of cacoxenite, a mineral phosphate

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Cacoxenite¹ is a widespread highly hydrated basic ferric oxyphosphate mineral distributed in gossans, iron- and manganese-bearing novaculites, and an occasional constituent of ferruginous soils and sediments. For long a troublesome aspect of mineralogical science and extensively investigated in the past, cacoxenite remained an enigma for lack of suitable material for X-ray diffraction single crystal study. We have now obtained a suitable specimen and report here that it has a framework structure containing enormous channels.

Cacoxenite elicited considerable interest due to its unusually high water content. A detailed study² of its multiple roles of

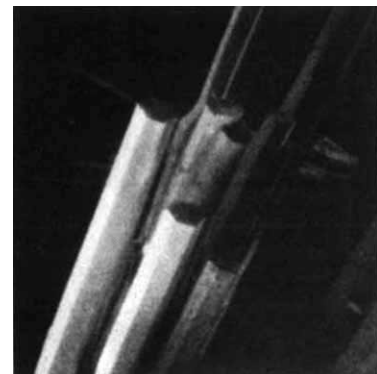


Fig. 1 Ga⁺ focused ion beam image of cacoxenite, Shady, Arkansas. Photograph width, 88 µm.

water yielded Fe₂O₃ 48.57 wt %, P₂O₅ 19.76%, H₂O(-) 18.69%, H₂O(+) 13.11% = 100.13% total. The H₂O(-) was determined on a sample from Eleonore Mine, Giessen, FRG, *in vacuo* with anhydrous sulphuric acid as desiccant; and H₂O(+) from ignition of the sample. Many other analyses have been published, the principal additional reported component being Al₂O₃. Our structure study yields ideally [AlFe₂₄O₆(OH)₁₂(PO₄)₁₇(H₂O)₂₄] · ~51 H₂O, the contents in brackets representing the structure's framework, and the remaining greater fraction of water evidently occurring in the enormous structural channels or pores. For this endmember formula, Al is in trigonal bipyramidal, Fe in octahedral and P in tetrahedral coordination by oxygen atoms. Computed contents of the ideal formula yield Al₂O₃ 1.10%, Fe₂O₃ 41.36%, P₂O₅ 26.04%, H₂O(-) 17.50%, H₂O(+) 14.00% = 100.00% total. Clearly, the agreement with the chemical analysis shows greatest discrepancy with the P₂O₅ content but the computed value is confirmed by earlier chemical analyses although these do not discriminate among the roles of water present. The refined single crystal data and the structure support *a* = 27.559(1) Å, *c* = 10.550(1) Å, *Z* = 2, space group P6₃/m. A similar cell was reported recently³ but this was determined from oriented fibres with a Weissenberg camera and the space group determination was inconclusive.

More than 10 yr was required to obtain crystals suitable for X-ray structure analysis. Despite examination of thousands of specimens in many localities, all crystals were far too thin, ranging from 1 to 20 µm in thickness, although lengths up to 1 mm were not uncommon. In addition, most samples displayed tufted or bunched aggregates of individual crystals which can be noted in the photograph taken from a scanning ion microscope in Fig. 1. Only one specimen, collected by one of us (P.B.M.) from Avant's Claim, near Shady, Polk Co., Arkansas, showed single hexagonal prisms of deep orange colour and embedded in amorphous ferric oxyhydroxide gel. The crystal used for structure analysis measured 60 × 60 × 100 µm and afforded superior single crystal data.

Over 4,000 symmetry independent reflections were collected on an automated Picker FACS-1 diffractometer. With scan speed 8° min⁻¹, base scan width of 2°, reflections to 2θ = 126° were collected from CuKα radiation owing to the small size of the crystal and the large *a* axis and primitive cell. The data were corrected for crystal absorption. Structure solution was assisted by direct methods built into our SHELX-76 program and led to an initial model which proved fruitful. The entire analytical phase of the study used the DEC VAX 11/780 computer facility at the University of Chicago. At *R* = 0.20, the water molecules (labelled W) directly associated with the framework and bonded to only one (Fe, Al) cation appeared. At an earlier stage, the oxygens not directly bonded to phosphorus were resolved. There were two types: four independent atoms each bonded to three iron atoms (labelled OX) and one atom bonded to two iron atoms only (labelled OH). Below *R* = 0.14, further scattering curve assignments could be made. The larger cations proved to be principally Fe in five independent

Fig. 2 The labelled asymmetric unit of the caxoxenite structure. Primed labels are related to Table 1 by inversion. Heights are fractional coordinates in z .

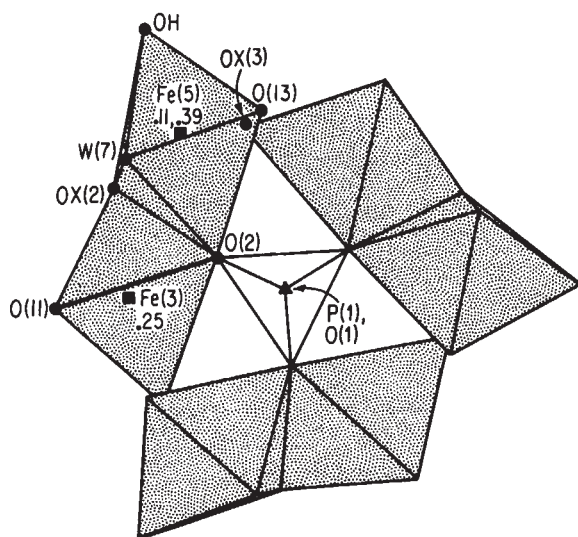
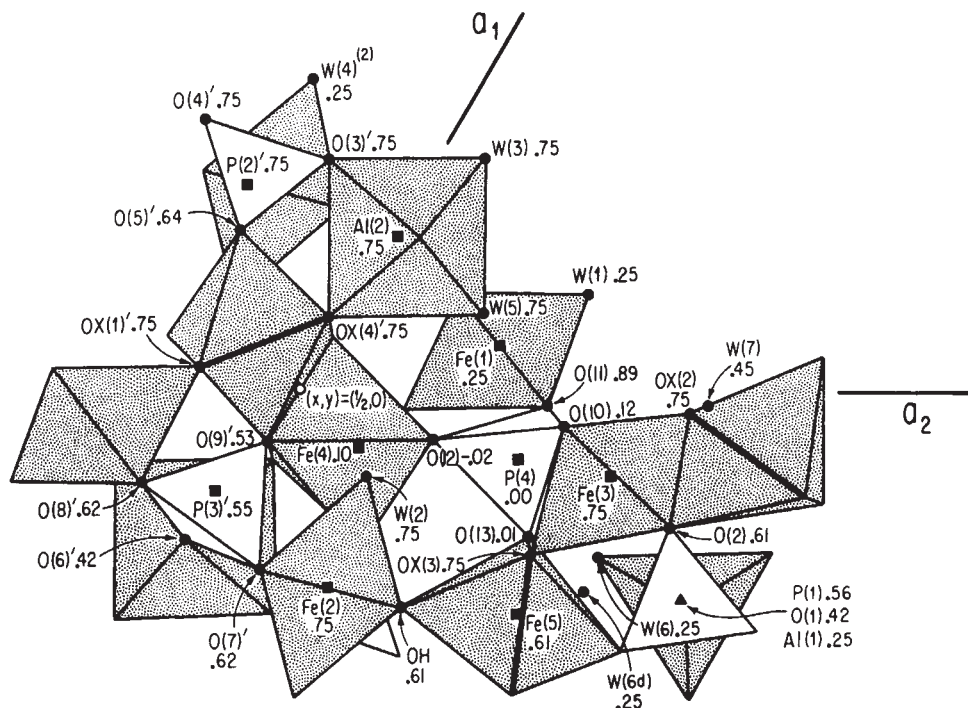


Fig. 3 The first building block in caxoxenite, much like the central girdle in the Keggin molecule. The centre is $(\frac{2}{3}, \frac{1}{3}, z)$.

positions and principally Al in two remaining independent positions. The O(4) associated with a PO_4 tetrahedron was not bonded to any other cation. However, our present model suggests it receives two hydrogen bonds from neighbouring water molecules and is probably O^{2-} , not $(\text{OH})^-$ as the formally charged species. The present proposed formula at $R = 0.118$ is $[\text{Al}(\text{Al}, \text{Fe})_3\text{Fe}_{21}\text{O}_6(\text{OH})_{12}(\text{PO}_4)_{17}(\text{H}_2\text{O})_{24}] \cdot \sim 51 \text{ H}_2\text{O}$. A labelled structure drawing of the asymmetric unit is shown in Fig. 2 and the atomic coordinates are presented in Table 1.

The pore water molecules will not be discussed here as further study is required to elucidate them. Polyhedral bond distance averages are $\text{Fe}(1)\text{--O}$ 1.98, $\text{Fe}(2)\text{--O}$ 2.01, $\text{Fe}(3)\text{--O}$ 2.02, $\text{Fe}(4)\text{--O}$ 2.04, $\text{Fe}(5)\text{--O}$ 2.01, $\text{Al}(1)\text{--O}$ 1.84 (AlO_5), $\text{Al}(2)\text{--O}$ 1.93 (AlO_6), $\text{P}(1)\text{--O}$ 1.53, $\text{P}(2)\text{--O}$ 1.54, $\text{P}(3)\text{--O}$ 1.54 and $\text{P}(4)\text{--O}$ 1.54 Å. In addition setting $\text{M} = \text{Fe}^{3+}$ and/or octahedral Al^{3+} : $\text{M--OX}(1)$ 1.90, $\text{M--OX}(2)$ 2.08, $\text{M--OX}(3)$ 1.91, $\text{M--OX}(4)$ 2.14 and M--OH 1.96 Å. We have assigned formal $\text{O}^{2-} = \text{OX}(1)$, $\text{OX}(3)$, and $(\text{OH})^- = \text{OX}(2)$, $\text{OX}(4)$, OH . Difficulty arises because of the plethora of hydrogen bonds, many of which have neither yet been located nor assigned, so a classical Pauling-type bond length-bond strength calculation is meaningless.

Table 1 Atomic coordinates and isotropic displacement factors for caxoxenite

Atom	x	y	z	$B(\text{\AA}^2)$
Fe (1)	0.4759 (2)	0.1140 (2)	1/4	0.8 (1)
Fe (2)	0.6542 (2)	0.0940 (2)	3/4	1.2 (1)
Fe (3)	0.5717 (2)	0.2330 (2)	3/4	0.8 (1)
Fe (4)	0.5400 (1)	0.0525 (1)	0.1025 (4)	0.7 (1)
Fe (5)	0.6758 (1)	0.2229 (1)	0.6066 (4)	0.8 (1)
Al (1)	2/3	1/3	1/4	0.3 (2)
Al (2)	0.3831 (4)	0.0107 (4)	3/4	0.1 (1)
P (1)	2/3	1/3	0.562 (1)	0.5 (3)
O (1)	2/3	1/3	0.422 (3)	0.5 (4)
O (2)	0.6060 (6)	0.2979 (6)	0.614 (2)	0.1 (1)
P (2)	0.6555 (4)	0.1129 (4)	1/4	0.2 (1)
O (3)	0.6731 (10)	0.0672 (10)	1/4	0.9 (4)
O (4)	0.7063 (10)	0.1709 (10)	1/4	0.8 (4)
O (5)	0.6203 (7)	0.1022 (7)	0.128 (2)	0.9 (3)
P (3)	0.4061 (2)	0.0107 (2)	0.049 (1)	0.1 (1)
O (6)	0.3839 (6)	0.0171 (6)	-0.077 (2)	0.1 (1)
O (7)	0.3591 (7)	-0.0398 (7)	0.119 (2)	1.1 (3)
O (8)	0.4256 (7)	0.0650 (7)	0.122 (2)	0.7 (3)
O (9)	0.4572 (6)	-0.0001 (6)	0.031 (1)	0.1 (1)
P (4)	0.5522 (2)	0.1657 (3)	0.000 (1)	0.2 (1)
O (10)	0.5239 (7)	0.1706 (7)	0.122 (2)	0.6 (3)
O (11)	0.5284 (6)	0.1817 (6)	-0.113 (2)	0.1 (1)
O (12)	0.5403 (6)	0.1053 (7)	-0.021 (2)	0.5 (3)
O (13)	0.6155 (6)	0.2062 (6)	0.015 (2)	0.2 (2)
OH	0.6680 (7)	0.1496 (7)	0.615 (2)	1.0 (3)
OX (1)	0.5144 (10)	0.0740 (10)	1/4	0.9 (4)
OX (2)	0.5172 (9)	0.2646 (9)	3/4	0.3 (4)
OX (3)	0.6294 (9)	0.2150 (9)	3/4	0.1 (1)
OX (4)	0.5520 (11)	0.0025 (11)	1/4	0.9 (4)
W (1)	0.427 (1)	0.156 (1)	1/4	1.8 (5)
W (2)	0.570 (1)	0.079 (1)	3/4	0.8 (4)
W (3)	0.325 (1)	0.034 (1)	3/4	2.3 (6)
W (4)	0.376 (1)	0.262 (1)	3/4	2.1 (6)
W (5)	0.443 (1)	0.094 (1)	3/4	1.6 (5)
W (6)	0.620 (3)	0.256 (3)	1/4	6.8 (17)
W (6d)	0.656 (4)	0.259 (3)	1/4	11.5 (20)
W (7)	0.510 (1)	0.269 (1)	0.047 (2)	1.2 (3)

Estimated errors in parentheses refer to the last digit.

The most intriguing feature of the caxoxenite structure is the enormous free diameter of the pore or channel which is oriented parallel to the c -axis. The minimum diameter is the circle tangent to the W(3) atom which is centred at the cell's origin. An effective radius of 1.40 Å was applied to the W(3) centroid. The calculated free pore diameter is 14.2 Å. The crucial test is now

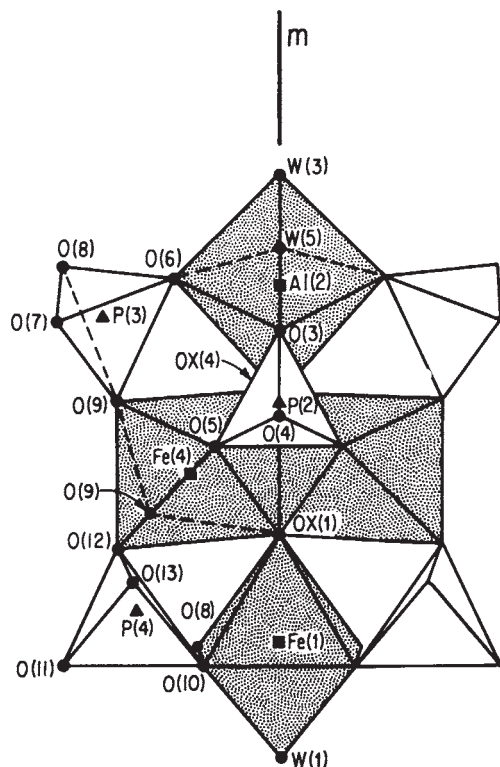


Fig. 4 The second building block in cacoxenite, an octahedral tetramer showing five corner-linking PO_4 tetrahedra. This unit polymerizes at the O(9)–O(9) edge to form an infinite bundle parallel to c which is oriented east–west.

to establish the loci of the first nearest-neighbour hydrogen-bonded water molecules and the stability of the framework as the pore water molecules are progressively removed.

The structure of cacoxenite's framework can be conceived as the fusion of two fundamental building blocks which are found as components in some other phosphate mineral structures. The first, Fig. 3 centred at $(\frac{2}{3}, \frac{1}{3}, z)$, and so on, is related to the central girdle in the Keggin molecule and to mitridatite, with composition $[\text{PFe}_6^{3+}\text{O}_{28}]$. Mitridatite, $\text{Ca}_6(\text{H}_2\text{O})_6[\text{Fe}_9^{3+}\text{O}_6(\text{PO}_4)_9] \cdot 3\text{H}_2\text{O}$, of similar paragenetic setting to cacoxenite and also an oxyphosphate; has 75 atoms in the asymmetric unit⁴ but it is a dense sheet structure, not a channel or pore structure. The second fundamental building block in Fig. 4 bears a striking resemblance to the octahedral tetramer in amarantite, leucophosphate and melonjosephite⁵. In cacoxenite, the tetramer actually shares edges defined by inversion to form infinite chains or bundles parallel to the c -axis. This chain may explain the acicular to columnar characteristics of the mineral, presumably through rapid growth or polymerization along the c direction. This second unit is situated in projection around the 2_1 -screw axes at $(\frac{1}{2}, 0, z)$, and so on. The entire chain is reminiscent of the motif in melonjosephite, a phase also noted for its fibrous characteristic parallel to its chain direction⁶.

Taken together, the two distinct fundamental building blocks define all but five of the 36 non-equivalent framework atoms in cacoxenite. The atoms which remain include Fe(2), Al(1), W(2), W(4) and W(6). Fe(2) is bonded to W(2) and W(4), and Al(1) to W(6) of this collection. The $\text{Fe(2)}\phi_6$ octahedron serves as a bridge between the two building blocks through OH and O(7). The $\text{Al(1)}\phi_4$ trigonal bipyramid is the sole bond to W(6). Incidentally, W(6) and W(6d) appear to be disordered but coupled to each other in a crystallographically complementary way, suggested by the short W(6)–W(6d) 0.96 Å separation. This is the only disordered region so far found in the cacoxenite framework. It is not known if $\text{Fe(2)}\phi_6$ serves as part of another building block which can be identified in other structures.

Is cacoxenite unique or does it represent just one of a family of phases based on octahedral–tetrahedral incompleated frameworks and large open channels, pores or cavities? Several clues suggest that other phases would be confined to low temperature of formation ($<250^\circ\text{C}$), slightly to markedly basic environments (presence of ferric oxyhydroxide gels, manganese oxides, other phosphates in a paragenesis involving hydroxyl- and oxy-anions) and would possess morphologies of an acicular nature. The acicular characteristic would arise from the appearance of corner- and edge-linked chains parallel to the axis of morphological elongation. Such phases may possess large structure cells and complex compositions since several distinct fundamental building blocks may be involved. A basic ferric oxyhydroxyphosphate would be expected to possess a deep orange to red-brown colour since the only chromophore is Fe^{3+} in octahedral coordination with respect to oxygens. If the phase is hexagonal in structure, a close mimetic relationship with cacoxenite would be expected, suggesting that cacoxenites should be routinely examined by X-ray diffraction to assure pedigree.

Acicular phases in such environments with very complex powder X-ray diffraction patterns including strong intensities at low-diffraction angle should be given special attention.

Environments where many inadequately described and poorly understood phases have been recorded involve low-temperature assemblages of oxidized ores such as at Chuquicamata, Chile, Franklin, New Jersey, and Cornwall, UK. The Cornish species liskeardite⁷, $\sim\text{Al}_2(\text{OH})_3(\text{AsO}_4) \cdot 3\text{H}_2\text{O}$, occurring as thin acicular crystals and possessing a complex powder pattern with strong intensities at low angle is presently being scrutinized further. Challantite, analogous to basic synthetic $\text{Fe}_{14}(\text{OH})_6(\text{SO}_4)_{18} \cdot 58\text{H}_2\text{O}$ (ref. 8) is another such example.

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Patterns of chemical change during bone fossilization

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The uptake of elements by bone during fossilization is a well-recognized phenomenon but the nature of, and the factors leading to, chemical heterogeneity in fossil bone are only just starting to be elucidated. Indeed, in the recent past the assumption that fossil bone is chemically homogeneous has been commonly used in several investigations including those of bone dating and dietary studies. In an attempt to understand the processes of element uptake we have investigated some chemical heterogeneities in a fossil bone and have assessed the potential use of bone composition as an indicator of the trace-

element contents of associated groundwater. Our study extends other work^{1,2} by giving the first analysis of rare earth element (REE) distribution in a fossil bone and by showing that information on the redox state and REE abundance pattern of the groundwater can be obtained. The results contribute to the interpretation of bone analyses in studies on provenance and age determination as well as in the diet and health of past populations.

Five bones from the Olduvai Gorge, Tanzania, and from elsewhere have been analysed. All five bones show similar but not identical chemical distribution patterns. Variations in the patterns could result from the exposure of bones to different environmental conditions². We have selected one bone as a typical example of the nature of element distribution found in the specimens that we analysed. This specimen is a mammalian bone with a thick, uniform cortex of secondary (Haversian) bone from site TK, Bed III, of the Olduvai Gorge. Sediment (sandstone) was adhering to the outer surface and was in the central core. Details of Bed III and of the site are given elsewhere³. The locality was chosen because it is in an area rich in alkaline igneous rocks so that the associated groundwaters are rich in fluoride and are likely to contain relatively high concentrations of REE and other trace elements suitable for this investigation.

A section across the bone cortex was cut into five parts, from the outer surface (piece A) to the inner part of the cortex (piece E); thicknesses and relationships are shown on Fig. 1. The outer matrix (sediment) and core material were also sampled. Each sample was analysed (single determinations) for the elements listed in Table 1, by instrumental neutron activation analysis; errors based on counting statistics are better than 3% (σ). X-ray diffraction showed that each bone piece consists of apatite and trace amounts of calcite; electron microprobe analysis revealed trace amounts of fluorite, baryte and Fe, Mn oxides as infillings in the osteone canals.

Table 1 lists the maximum concentration of each element in the bone and relative to the exterior sediment (enrichment factor). Most elements show a significant, but not always systematic, decrease in concentrations from the exterior to the interior parts of the cortex, the extent of the variation is given in Table 1 as the maximum:minimum concentration ratio. The patterns of element variation can also be grouped, as a means of data presentation, into three broad types (Table 1), typical examples of which are depicted in Fig. 1.

Our data show significant variations in trace-element concentrations across the bone (up to a factor of >700, Table 1), as has been observed in other cases², and despite the use of rather large radial distance increments (Fig. 1). The variations are likely to be larger if smaller increments across the bone were to be considered². The wide extent of the variation indicates that the process of element exchange and equilibration takes place at different rates for different elements. Modern bones collected from the Serengeti Plain near the Gorge have also been examined. The REE and most other trace elements are at concentration levels which are insignificant compared with the values recorded here, except for Sr and Ba which are at concentrations up to one third of the maxima found in the fossil bone.

Parker and Toots¹ classified the minor elements in fossil bone into three categories: (1) Those that are incorporated into bone apatite during the life of the animal (for example, Na, Sr). (2) Those that undergo postmortem enrichment as functions of element availability and time (for example, F, Y). (3) Those that undergo postmortem enrichment as a function of the nature of the diagenetic environment (for example, Si, Mn, Fe). Our findings suggest that some sodium is added to the bone through postmortem enrichment, as there is a higher concentration of this element in the exterior relative to the interior parts of the cortex (compare with Sr, but note its high concentration). Most of the other elements studied here fall solely into the second category defined by Parker and Toots¹ but with the notable exception of variable valency cations, such as Ce, which will be

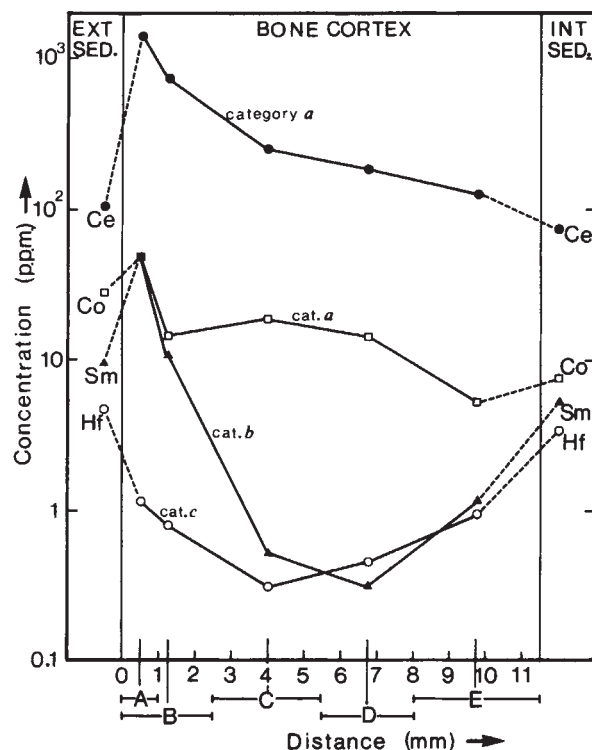


Fig. 1 Concentration variation with depth (mm) in the fossil bone. The values are plotted at the median of the radial distances of each bone piece. Concentrations in the two sediment samples are also plotted. The pattern categories, or types, are described in Table 1.

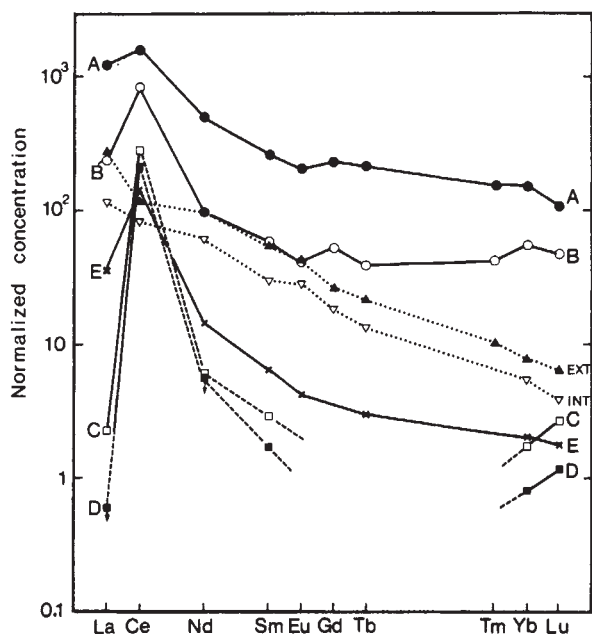


Fig. 2 Chondrite-normalized⁷ REE abundance plot for each piece of bone and of the two sediment samples (dotted lines). Dashed lines are used where an element concentration was below the limit of determination (indicated by an arrow) or where insufficient data are available.

affected by the redox conditions of the diagenetic environment; these fall into both categories (2) and (3). These results suggest that such element categorization is an oversimplification as a description of bone composition and alteration. Furthermore they show that Th and U concentrations have changed with time and any measured age derivable from isotope ratio⁴ or fission track studies⁵ could be affected. Large-scale variations

Table 1 Trace-element concentration data for an Olduvai bone

	Maximum concentration* (p.p.m.)	Maximum enrichment factor	Concentration variation (max/min)	Pattern type†
Na	13,870	0.53	3.0	a
Sc	29.8	5.5	1.7	a
Cr	23	0.33	>6	a
Co	49	2.4	9.3	a
Ni	<50	<0.14	—	—
Rb	<10	<0.15	—	—
Sr	9,980	2.1	1.1	Flat
Sb	0.65	>2.6	2.5	—
Cs	<0.5	<0.74	—	—
Ba	5,200	0.21	2.2	a?
La	412	4.5	>390	b
Ce	1,409	13.7	11	a
Nd	295	5.0	>74	b
Sm	48	5.0	160	b
Eu	14	5.1	>700	b
Gd	58	8.8	>30	b?
Tb	9.9	10.0	>100	b
Tm	4.7	15.2	>47	b?
Yb	31	20	190	b
Lu	3.7	17.4	91	a
Hf	1.1	0.24	3.7	c
Ta	<0.3	<0.07	>3	c?
Th	210	17.8	>400	b
U	12	21	3.0	a

* The maxima are in the outermost piece (A) except in the case of Sb (piece B)

† a, A trend of decreasing concentration from the exterior to the interior, with relatively low concentration variation values. b, A sharp decrease in concentration from the exterior to piece D with an increase to higher concentrations in piece E (innermost). c, An approximately U-shaped variation. Four elements could not be assigned to a pattern type because either there were insufficient data (Ni, Rb, Cs) or the variation is random (Sb).

in U concentrations across some fossil bones have been recorded elsewhere^{2,6}.

The distribution of the REE has proved helpful in the elucidation of the process of element exchange. Their chondrite-normalized⁷ plots (Fig. 2) show the wide range in abundances across the bone cortex but each plot has a similar form with relative enrichment in the light REE and a tendency for a uniform heavy REE distribution. Figure 2 also shows the plots for the exterior and interior sediments; both are similar but the interior sediment has slightly lower REE contents.

The broad similarities in the REE concentration profiles and in the chondrite-normalized plots for the bone suggest that the abundance ratios directly reflect the REE abundance ratios in the exchange medium. This medium is more likely to be groundwater than the enclosing sediment, since the REE abundance levels in the bone pieces (A and E) adjacent to the inner and outer sediments are very different and have subtle but significant differences in their normalized plots (Fig. 2) despite the similarity in the two patterns for the sediment. Provided that REE distribution coefficients between bone and an appropriate aqueous solution are known, it should be possible to use the outer portion of such bone samples as an indicator of the REE abundance pattern of the associated groundwater during fossilization. However, the use of bone in this way could only be realized if there was independent evidence to show that the specimen had been in contact with only one groundwater system during the fossilization period.

The behaviour of Ce supports the case for groundwater–bone interaction. A positive Ce anomaly is present in the pattern for each piece of bone, the extent tending to decrease with increasing total REE contents (Fig. 2). The sediment contains no significant cerium anomaly and, therefore, cannot have contributed to the excess Ce in the bone. The Ce concentration profile across the cortex is also distinct from that of the other REE (Fig. 1 and Table 1). These aspects are explicable if some or all of the Ce

in the associated groundwater was in a higher oxidation state (+4) than the other REE ions (+3); this would lead to different equilibrium distribution coefficients for groundwater–bone–apatite systems and different diffusion rates in the bone. The variation in the Ce anomaly (Fig. 2) could result, therefore, from faster rates of uptake and equilibration of Ce⁴⁺ relative to the trivalent REE. In this case the bone composition is acting as an indicator of the oxidizing potential of the associated groundwater⁸. The nature of the Ce anomaly cannot be explained solely by an excess Ce³⁺ abundance in the associated groundwater. The absence of an Eu anomaly (Fig. 2), which could be caused by the presence of Eu²⁺ in reducing conditions, is in keeping with the presence of quadrivalent cerium.

Element exchange between fossil bone and groundwater is generally a low-temperature process. Chemical heterogeneities of the type considered here indicate that the process is often slow and could continue over a considerable time. Hence, before the isotopic or chemical composition of a bone can be used to make conclusions concerning its age or history it is essential to have prior information on the element distribution and the nature of the associated groundwater.

We have shown that chemical exchange between a bone and its environment, during fossilization, leads to compositional heterogeneities in the form of systematic concentration gradients for several elements. The resultant patterns, especially in the case of the REE, could be used to give an insight into the oxidizing conditions and the trace-element abundance ratios of the associated groundwater.

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Climatic control of turbidite deposition on the Madeira Abyssal Plain

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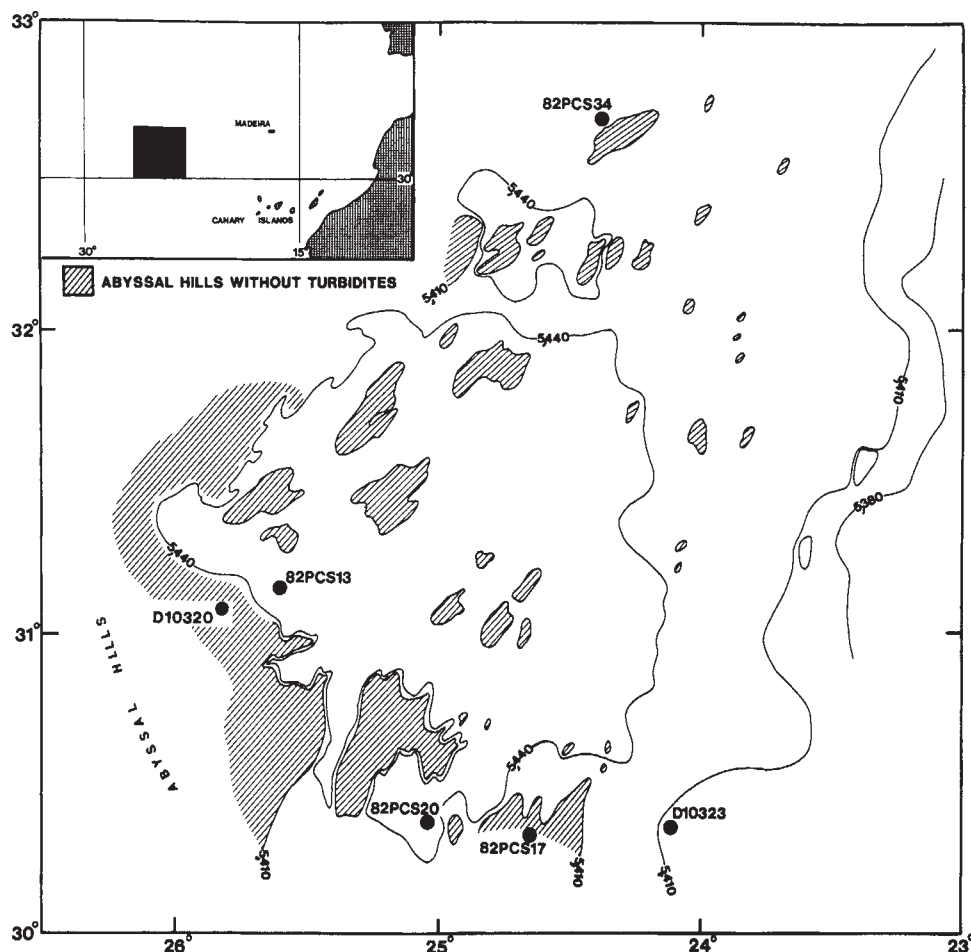
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Studies of sedimentation in the southwestern part of the Madeira Abyssal Plain have revealed sequences of turbidites correlatable over thousands of square kilometres. Turbidites are sediments transported by turbidity currents and represent rapidly accumulated sedimentary units¹, in this case in the distal part of the flow they are very fine-grained and homogeneous, occasionally with silty bases. Individual turbidites are separated by thin pelagic units which alternate from oozes to pelagic clays. A stratigraphy for these pelagic units has been produced from combining coccolith distributions and lithological changes, which enables comparison to cores containing only pelagic sediments from outside the turbidite province. These comparisons show that turbidity currents cause no significant erosion in the area. The major turbidites occur between lithological alternations which reflect global climate changes associated with glaciations and related eustatic sea-level fluctuations. Turbidites have been linked with eustasy on previous occasions, but only with low stands of sea level^{2,3}. Our work shows that turbidites are more likely to occur during regressions and transgressions rather than in the quiet periods between.

Fig. 1 Detailed bathymetry of study area with locations of core sites. Shaded areas represent abyssal hills with limited or absent turbidites.



Research into the feasibility of disposing heat emitting radioactive wastes has included investigations in the southwestern part of the Madeira Abyssal Plain. The sediments in this area consist of very fine-grained (2–35 μm) distal turbidites with calcium carbonate contents of 50–60%. The turbidites are comprised of predominantly the 'e' division of Bouma's turbidite facies model⁴, occasionally with thin basal units of laminated silt belonging to the 'd' division. Source areas of these turbidites are the north-west African continental slope and probably also the slopes of the Madeira and Canary archipelagoes. Individual turbidites range from a few centimetres to several metres thick and are generally separated by several-centimetre thick layers of non-turbiditic pelagic sediment. These pelagic sediments vary from oozes to pelagic clays as the area lies in the lysocline where varying rates of calcium carbonate dissolution occur depending on the corrosiveness of the bottom water and rate of supply of carbonate material^{5–7}. Dissolution has been particularly severe during glacial periods when the area lay below the CCD (calcium carbonate compensation depth) where rate of corrosion of calcium carbonate exceeds rate of supply, thus producing pelagic clay. During interglacials dissolution was considerably reduced giving rise to marls or marly oozes. In slightly shallower areas nearby, cores show less severe effects of dissolution with alternations between marly ooze and marls (Figs 1 and 2 core D10320). In phase relationships between dissolution and palaeoclimatic records have recently been shown in North Atlantic cores⁶ back to at least 150,000 yr.

Cores D10320 and D10323, which consist predominantly of pelagic sediment, provide good reference sections for a combined bio/lithostratigraphy. This stratigraphy has been compared with oxygen isotope stages^{8,9}. It is stressed that direct oxygen isotope measurements in these cores are not reliable due to the slow deposition rate and severe dissolution in some intervals. The main elements of the coccolith stratigraphy are: dominance of *Emiliania huxleyi* since late oxygen isotope

stage 5¹⁰, a dominance of small *Gephyrocapsas* (mainly *G. aperta*) during oxygen isotope stages 6 and 7¹¹, a dominance of *Gephyrocapsa caribbeanica* below oxygen isotope stage 7¹⁰, and the extinction of *Pseudoemiliania lacunosa* in oxygen isotope stage 12¹⁰. Within this framework the lithological changes, observed within the various coccolith zones, are used to give the exact oxygen isotope stage boundaries. Any missing zone can be clearly identified as there will be less than the required number of lithological units between any two coccolith datums. The combined coccolith and lithological stratigraphy indicates that cores D10320 and D10323 extend to oxygen isotope stages 13 and 10 respectively (Fig. 2).

To compare the rates of deposition of pelagic sediment in the turbidite cores (82PCS13, 82PCS20 and 82PCS34) with that in the non-turbidite cores (D10320 and D10323), the pelagic intervals in these cores have been plotted in Fig. 2 without the intervening turbidites. Cores 82PCS13 and 82PCS20 show complete suites of oxygen isotope stages back to stages 6 and 7 respectively, whilst core 82PCS34 shows a record to beyond stage 11 with oxygen isotope stages 2 and 8 missing. The reasons for these two missing glacial intervals are uncertain, but by comparison with cores D10320 and D10323, both would be very thin, and they may have been removed by locally increased current activity around the adjacent hill (Fig. 1). Increased current strengths have been recorded around small abyssal hills¹² and such currents may have been stronger during glacial intervals. Comparisons of pelagic sediment deposition in cores 82PCS13 and 82PCS20 with D10323 (taken at approximately the same water depth) in Fig. 2, show slightly higher sedimentation rates in the turbidite cores, but this may be due to bioturbation of turbidite material up into the pelagic units. Certainly there is no evidence of erosion of the pelagic intervals in these two cores by incoming turbidity currents. Core 82PCS34 shows slightly slower deposition rates than the other cores and lacks stages 2 and 8.

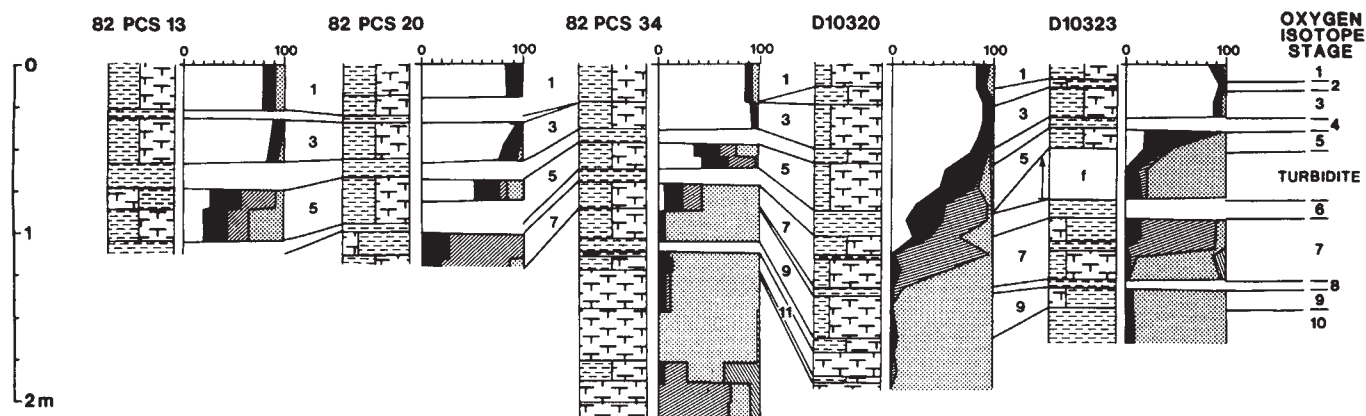


Fig. 2 Pelagic intervals of turbidite cores 82PCS13, 82PCS20 and 82PCS plotted without intervening turbidites and compared with pelagic cores D10320 and D10323. Coccolith stratigraphy plotted as cumulative percentages of five coccolith species. Blank sections due to complete dissolution. Oxygen isotope stage boundaries placed within the constraints of the coccolith stratigraphy at lithological changes.

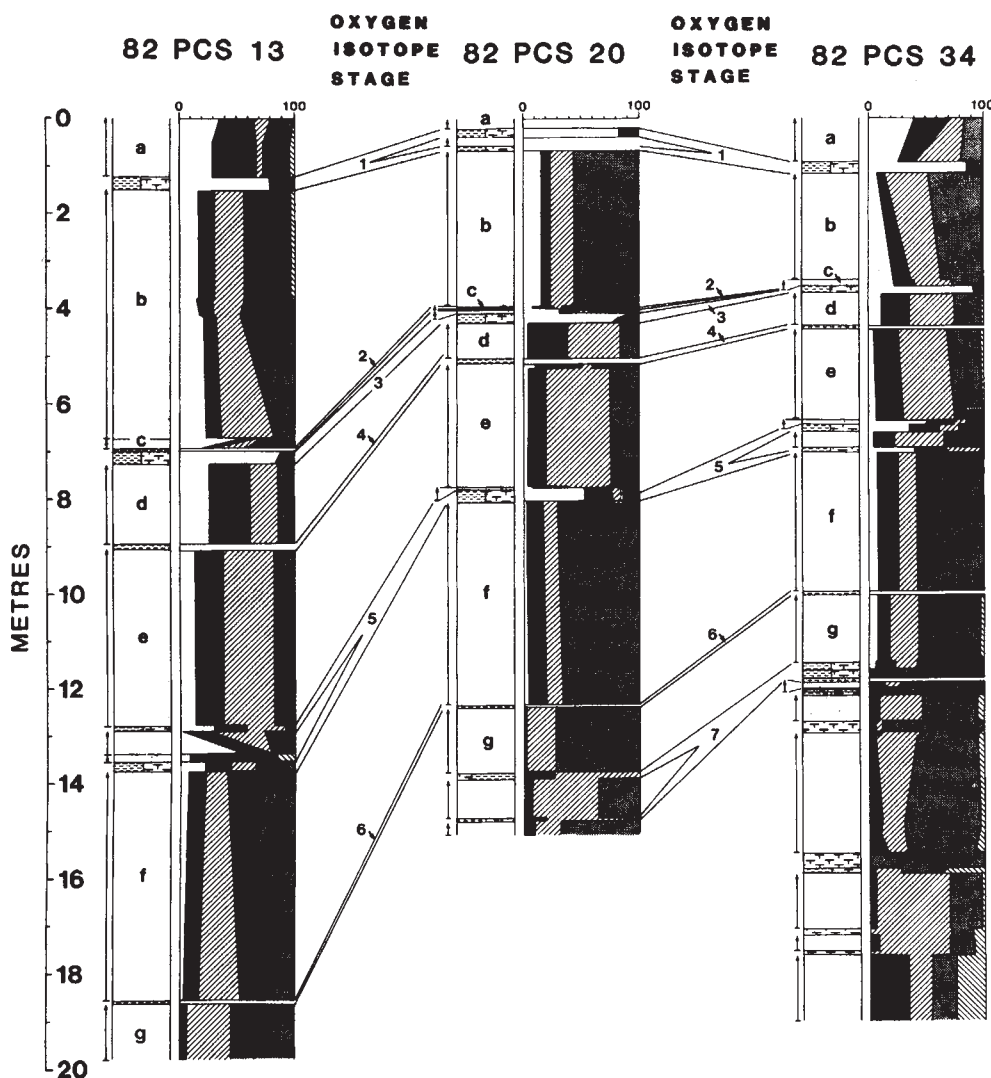


Fig. 3 Correlation of turbidite cores using correlation lines as derived in Fig. 2. Turbidites present in all three cores are lettered consecutively from the top.

When the turbidites are placed back into the core plots (Fig. 3) the correlations derived in Fig. 2 can be reapplied and we can see which turbidites correlate with each other. Coccolith floras of individual turbidites are fairly constant both within a core and between cores, whilst different turbidites have different signatures and can thus be easily identified at each site by the relative proportion of species present. Other parameters such as organic carbon contents, diatom floras¹³ and magnetic intensities¹⁴ all substantiate the correlations made in Figs 2 and 3. The major turbidites can be found in each core whilst the thin turbidites are not consistently present.

The pelagic lithologies in the cores are almost invariably different at the top and base of each turbidite for example, turbidite 'd' has marl above and pelagic clay beneath, whilst turbidite 'e' has clay above and marl beneath. Because we have evidence that the turbidity currents cause no significant erosion of the pelagic sediments in the area where the cores were collected, the turbidites must be deposited during the transition from one sediment type to another. These transitions reflect global climate changes between glacial and interglacial conditions^{6,7} and thus major turbidite deposition in this area is associated with climate change. The most likely association is through eustatic sea-level changes. Correlation between the rate, and relative amount of, sea-level rise and fall with oxygen isotope stage boundaries¹⁵ show that the most major changes occur at the boundaries between oxygen isotope stages 5/6, 4/5 and 1/2. These boundaries coincide with the three largest turbidites in the cores. Less severe sea-level changes occur at the stage 3/4 and 2/3 boundaries¹⁵ and in these cases a turbidite is associated with the 3/4 stage boundary, but not with the 2/3 stage boundary. The more minor sea-level changes, within stage 5¹⁵, are associated with the oxygen isotope subdivisions of this stage¹⁶. In the turbidite cores two minor turbidites occur during stage 5 between the major turbidites 'e' and 'f' in 82PCS34. Core 82PCS20 received no mid-stage 5 turbidite, but it did receive a mid-stage 7 turbidite. Cronin did not consider sea-level changes earlier than stage 6, but a short-lived period of rapid ice growth has been postulated during stage 7¹⁷, which would be associated with sea-level fluctuations. Again this turbidite is not present throughout the area, thus there may be some relationship between magnitude of sea-level change and the scale and intensity of turbidity flow processes. Below stage 7 in core 82PCS34 the association of turbidites with lithological boundaries is not so positive, no turbidite occurs between stages 9 and 10, for example. The reasons for this are unclear and as we have only one turbidite core through this interval we can draw no conclusions at present.

These deep-sea turbidites are thought to originate on the upper continental slope off north-west Africa or possibly from the Madeira Rise. Some of the turbidites contain relatively high organic carbon contents (>1%) which suggests initial deposition in areas of relatively high productivity such as the shelf or upper continental slope. A series of submarine slides and debris flows have been mapped along the north-west African continental margin^{18,19}. These are all of considerable magnitude and could be responsible for initiating the turbidity currents discussed here. The Saharan slide has been dated as 15,000–16,000 yr BP (ref. 18) which corresponds to the initial rapid early deglaciation at the end of oxygen isotope stage 2, dated as 16,000–13,000 yr BP (ref. 20). The large-scale sediment mass movements on the upper continental slope off north-west Africa, concluded to initiate turbidity flows on the Madeira Abyssal Plain, thus seem to be triggered during major sea-level rises and falls and probably do not operate at random as previously reported¹⁸.

Eustasy has been proposed as a controlling mechanism for turbidity current initiation^{2,3}, but only in that such currents would be preferentially produced during low stands of sea level. However, our work suggests that, at least in the Upper Quaternary succession of the Madeira Abyssal Plain, major turbidity currents are initiated only during transgressions and regressions and not during the quiet intervals between. An apparent link exists between the magnitude of eustatic sea-level change and

the scale and intensity of turbidity flow which is associated with sediment instability on the north-west African continental slope.

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A physical mechanism for establishing algal populations in frazil ice

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In polar regions ice algal communities are not only conspicuous but may also be important production sites and sources of seed populations for pelagic communities^{1–3}. Except for some studies near land-based stations^{1,4–7}, there are few long-term observations of ice algal populations, and few studies have considered how they form and develop. Most researchers suggest that ice algae are derived from sparse water column assemblages that become trapped during ice formation; ice algal populations then develop through *in situ* growth¹. Some previous observations have suggested that ice crystals forming in the water column concentrate algal cells^{6–8}. Dense algal populations in young sea ice may have been recognized as early as the mid-nineteenth century, since Hooker (cited by Alexander²) described discoloured brash and pancake ice ("... a pale ochreous colour"). But until now, neither the mechanism for this harvesting nor the effects on the composition of the ice community has been clearly demonstrated. In the Weddell Sea, we have sampled young sea ice discoloured by algae, and we present evidence that the algae were concentrated by a physical mechanism. We explain how such a process may accumulate planktonic forms in ice communities.

We collected samples of young sea ice and samples from the water column along the ice edge during February and March 1980⁹. Ice samples were first melted and then processed as water samples. To estimate algal biomass we measured chlorophyll *a* of extracted pigments using a fluorometric method; we also measured nutrients and salinity in both ice and water by standard oceanographic procedures (data not shown). We examined algal populations with light and scanning electron microscopy to determine community composition.

Table 1 Chlorophyll *a* concentrations in young sea ice and concentrations in the water column, a prediction of the time (*t*) that would be required for algal populations in ice to have developed from those in the water and nutrient: salinity ratios

	Chl <i>a</i> (mg m ⁻³)	<i>t</i> (days)	N:S
Water column:	0.5 ± 0.3*	0	0.7
Young sea ice samples:	26.8	7.6	—
	21.6	7.2	0.5
	21.3	7.2	0.7
	13.4	6.3	0.2
	12.1	6.1	0.5
	11.7	6.0	—
	10.1	5.9	—
	8.3	5.7	0.7
	7.8	5.2	0.7
	7.6	5.2	0.3
	7.2	5.1	—
	6.7	5.0	0.7
	5.8	4.7	0.6
	5.8	4.7	0.8
	5.8	4.7	0.3
	5.3	4.5	0.2
	5.3	4.5	0.7
	4.2	4.1	0.7
	4.0	4.0	—
	3.8	3.9	0.7
	3.4	3.7	0.8
	2.7	3.2	0.7
	2.1	2.7	—
	1.2	1.7	0.3
	0.3	—	—
	0.3	—	0.7

t Was calculated from a logistic growth equation using 0.75 doublings per day as a maximum growth rate. N:S is the ratio of NO₃+NO₂ μmol l⁻¹ to salinity (0/00).

* Mean ± s.d. of 6 surface water samples from ice edge stations.

Table 2 The relative abundance (%) of diatom species in the water column and in young sea ice

Species	Water (10 samples)	Young ice (25 samples)
<i>Nitzschia cylindrus</i>	29 ± 19	40 ± 16
<i>Nitzschia curta</i>	25 ± 10	36 ± 18
<i>Nitzschia closterium</i>	8 ± 4	4 ± 4
<i>Chaetoceros dictyota</i>	7 ± 11	1 ± 1
<i>Nitzschia subcurvata</i>	6 ± 7	1 ± 1
<i>Nitzschia turgiduloides</i>	6 ± 8	1 ± 1
<i>Chaetoceros gracile</i>	3 ± 4	4 ± 8
<i>Nitzschia kerguelensis</i>	3 ± 3	2 ± 2
<i>Thalassiosira</i> sp.	2 ± 1	+
<i>Nitzschia sublineata</i>	1 ± 2	2 ± 3
<i>Chaetoceros bulbosum</i>	1 ± 1	+
<i>Dactyliosolen antarcticus</i>	1 ± 2	1 ± 1
<i>Corethron criophilum</i>	1 ± 1	2 ± 1
<i>Nitzschia angulata</i>	1 ± 2	1 ± 2
<i>Amphiprora kjellmanii</i>	1 ± 1	1 ± 1
<i>Nitzschia</i> sp.	+	1 ± 1
<i>Nitzschia neglecta</i>	+	+
<i>Tropidoneis fusiformis</i>	+	1 ± 1
<i>Rhizosolenia alata</i>	+	+
<i>Tropidoneis gausii</i>	+	1 ± 1

Values are average ± s.d.

The young sea ice samples were collected from small floes (~1 m diameter) with upturned edges (pancake ice) and ice sheets forming at the edges of open water (leads). Pancake ice forms by continuous accretion of ice crystals onto the sides of small floes¹⁰. In leads, ice crystals are advected downstream by wind-induced circulation, resulting in thicknesses exceeding 50 cm in less than 24 h¹¹. Previous studies^{10,12} have indicated ice growth rates of about 1 cm h⁻¹ or greater for open water and for areas with very thin ice. Since our samples were less than 24 cm thick and both types of ice were growing at rates typical for open water, 24 h is a conservative estimate of the age of this ice.

Chlorophyll *a* concentrations in these samples were up to 50 times greater than those in surface water (Table 1), which seemed too concentrated to be explained by *in situ* growth. We used Eppley's¹³ relationship of growth rate and temperature, $\log_{10}(\mu) = 0.0275 \times T - 0.07$, to estimate maximum growth rate (0.75 doublings per day at $T = -1.9^\circ\text{C}$) and calculated that the algal densities we found could require up to 8 days to develop from water-column levels (see Table 1). Since Palmisano and Sullivan¹⁴ have measured lower growth rates (0.42 doublings per day) for ice algae, our estimates of the time required to accumulate algal biomass may be too low, and our analysis therefore indicates that the populations in young sea ice must have been accreted by processes other than growth.

Nutrient data also support this idea. Assuming that ion ratios in ice remain constant at temperatures near -1.9°C , nutrient: salinity ratios in the water column and in ice should remain constant if the only process affecting nutrient concentrations was brine rejection. Algal growth would lower nutrient: salinity ratios by removing nutrient ions; however, NO₃+NO₂: salinity ratios (see Table 1) were not significantly different between ice and water ($t = 1.8$, $n = 23$, $P > 0.05$; two-tailed test). Furthermore, there was no correlation between these ratios and algal biomass in the ice (Pearson's $r = -0.12$, $P > 0.10$).

Comparing algal population composition in young sea ice with that in water samples suggests a non-selective concentrating mechanism. Diatom assemblages in the two environments were similar in species composition and relative abundance (Table 2, Fig. 1), although absolute cell concentrations in the ice were much higher than in the water. Moreover, non-growing forms such as archaeomonads were about as concentrated as apparently vegetative algal cells or chlorophyll *a*, which again suggests mechanical accumulation.

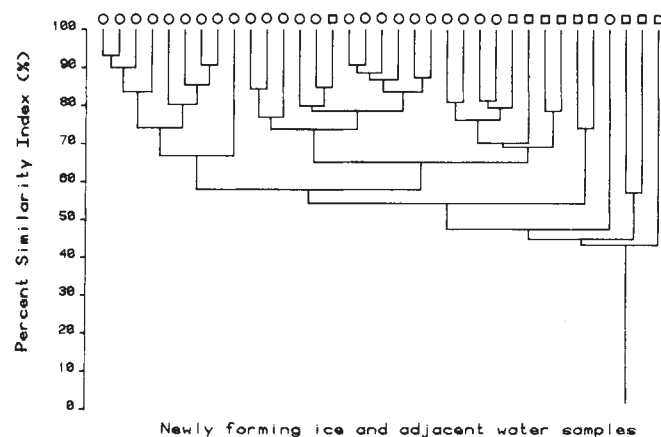


Fig. 1 A dendrogram showing the similarity of algal populations in (○) young sea ice and (□) adjacent water column samples. We calculated the similarity between stations using Whittaker's percent similarity index¹⁹ and used a group averaging, hierarchical, agglomerative clustering analysis to group similar samples. The average similarity between a composite assemblage of ice samples and individual member samples were $74 \pm 9\%$ ($n = 25$); this similarity was not significantly lower ($71 \pm 10\%$; $n = 35$) when both ice and water samples were combined (one-tailed *t*-test: $t = 1.45$, $P > 0.05$).

We propose that these algal populations were physically concentrated during frazil ice formation. Frazil ice develops as suspended crystals in turbulent waters, and because of its surface properties, particles in the water column adhere to the crystals; the harvesting of sediments and other material by frazil ice is well known¹⁵. Ackley¹⁶ suggests that algal cells could also be concentrated if crystallization occurred preferentially around algal cells. He calculated that such preferential nucleation could account for concentrations up to 10^6 cells per litre, and he even reports observing some cells within ice crystals. But as we found algal densities greater than 10^7 cells per litre in young ice, we believe that the most important concentrating mechanism is the harvesting of algal cells by frazil ice crystals rising to the surface to form new sea ice.

Physical accumulation may help explain some regional differences among ice algal populations. For example, ice algal populations are restricted to bottom or surface layers in many polar areas¹⁻³ but are found throughout the ice floes of the Weddell Sea³. Frazil ice generally characterizes the initial stages of sea ice growth; most floes then thicken because congelation ice forms under an established ice sheet at the ice-water interface¹⁷. The landfast ice floes at McMurdo Sound are composed primarily of congelation ice¹⁸ and algae are largely restricted to the bottom few centimetres¹⁴, or they develop in an aggregation of under-ice platelets⁷. In contrast, studies of Weddell Sea floes suggest that frazil ice production is continuous there: even older floes comprise mostly frazil ice¹⁸. Continuous frazil ice production and concurrent harvesting of algal cells may well account for the algae within floes from this region.

Populations accumulated by frazil ice are likely to be heterogeneous because frazil ice forms in patchy environments such as leads, polynyas, and along the ice edge where conditions are turbulent¹⁵. Certainly the young sea ice we saw was not discoloured uniformly. The variability of algal populations throughout older ice floes may reflect both ice-forming events and temporal changes in the species composition of water-column assemblages while the floes are growing.

Unlike ice populations in other polar regions, internal populations may not be available to metazoan grazers. But because they are trapped, they remain in floes throughout the year. Some of the algal cells may subsequently grow *in situ*; others may be consumed by similarly trapped heterotrophic protozoans, or bacteria may use the extracellular products of active cells and decompose dead ones. These populations and associated detritus are apparently released into the water column throughout the spring and summer and may thus provide a predictable and important food source for pelagic consumers³. Moreover, resting stages of pelagic forms are incorporated yearly into sea ice (J. G. Mitchell & M. W. Silver, personal communication); when released, these stages and surviving vegetative populations may constitute an important inoculum for pelagic populations.

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Lainyamok, a new middle Pleistocene hominid site

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It is generally believed that, during the middle Pleistocene in Africa, archaic *Homo sapiens* arose from *Homo erectus* and Acheulian tool industries were replaced by Middle Stone Age industries. The details of these transitions are poorly known because of the paucity of associated hominid, faunal and archaeological remains in datable contexts^{1,2}. We describe here Lainyamok, a new middle Pleistocene site. Fossil material includes three hominid teeth attributed to *Homo* sp. and a diverse fauna represented by unusually complete skeletal material. Acheulian artefacts are found both within bone clusters and thinly scattered throughout the site. The clustering of associated or articulated partial skeletons and artefacts suggests that Lainyamok may preserve evidence of the killing or butchering of individual, small- to medium-sized animals. Such sites have not previously been identified in the Pleistocene.

Lainyamok lies ~8 km north-west of Lake Magadi in Kenya (Fig. 1a, b). Fossils and artefacts were collected by Phillip Leakey and a team led by Kamoya Kimeu; a team led by the late W. W. Bishop undertook preliminary dating and geological mapping³. We report our preliminary analysis of the fossils and artefacts in the context of the geological data.

The Lainyamok fossil locality lies in a deep (23 m) graben that narrows and shallows southwards, deepening to the north. It is one of a series of grabens formed during grid-faulting of the southern Kenya rift valley, which probably predated sediment accumulation^{3,4}; faulting episodes also occurred during and after sedimentation^{5,6}. Thinning of fossiliferous sediments at Lainyamok towards the south and east suggests that the graben floor dipped to the north-west at the time of deposition.

The Lainyamok graben opens into a broad plain bounded on the west by a major fault scarp and to the east by a gently westerly-tilting block of the Kordjya Basalt, a thick, widespread basalt that forms the floor of the southern Kenya rift⁵. Overlying the basalt are the Magadi (or Plateau) trachytes, dated elsewhere from 1.4 Myr to 0.7-0.9 Myr⁶⁻⁹. Trachyte samples yield both normal and reversed polarities and may span the Jaramillo event (dated to about 0.89 Myr) in the late Matuyama epoch^{3,6,9}.

The sediments at Lainyamok were identified by Bishop³ as a thin, outlying development of the Oloronga Beds described by Baker³. The evidence for this view is: (1) Magadi trachytes underlie the Oloronga Beds and the sediments at Lainyamok; (2) both the Oloronga Beds and the Lainyamok sediments are lacustrine in origin, similar in colour and grain size, and accumulated in grabens associated with grid-faulting in the rift floor^{3,4,11,12}; (3) the type Oloronga Beds are capped by a thick, pisolithic caliche, as are the Lainyamok sediments¹⁰. An obsidian flow within the Oloronga Beds is dated to 0.78 ± 0.04 Myr⁸. The youngest part of the Oloronga Beds is believed by Eugster^{4,10} to be about 0.4 Myr, although they may be as young as 0.1 Myr.

Table 1 Lainyamok faunal list

Geological bed	Taxon		
Capping calcrete	Artiodactyla	Suidae	indet.
Rootcast silts	Pisces		cf. <i>Tilapia</i>
	Artiodactyla	Bovidae	<i>Gazella</i> sp.
	Perissodactyla	Equidae	<i>Parmularius</i> sp.
	Carnivora	Canidae	indet.
Khaki tuff	Aves	Struthionidae	<i>Struthio</i> sp. (eggshell)
	Reptilia	Phidia	indet.
	Primates	Cercopithecidae	<i>Papio</i> sp.
	Lagomorpha	Leporidae	<i>Lepus</i> cf. <i>capensis</i>
	Rodentia	Thryonomyidae	<i>Thryonomys</i> cf. <i>swinderinus</i>
		Pedetidae	<i>Pedetes</i> cf. <i>caffer</i>
		indet.	
	Carnivora	Canidae	cf. <i>Canis</i> sp.
		Hyaenidae	<i>Crocota</i> sp.
		Felidae	<i>Felis</i> cf. <i>libyacus</i>
			<i>Felis</i> sp.
			<i>Panthera</i> cf. <i>leo</i>
			<i>Orycteropus</i> <i>afer</i>
	Tubulidentata	Orycteropidae	indet.
	Proboscidea	Elephantidae	<i>Procapra</i> cf. <i>capensis</i>
	Hyracoidea	Procaviidae	indet.
	Perissodactyla	Equidae	<i>Phacochoerus</i> sp.
	Artiodactyla	Suidae	<i>Giraffa</i> sp.
		Giraffidae	<i>Parmularius</i> sp.
		Bovidae	<i>Gazella</i> sp.
			<i>Hippotragini</i> sp. indet.
Cellular rock	Pisces		cf. <i>Tilapia</i>
Pink/brown silts	Mollusca		<i>Limicolaria</i> sp.

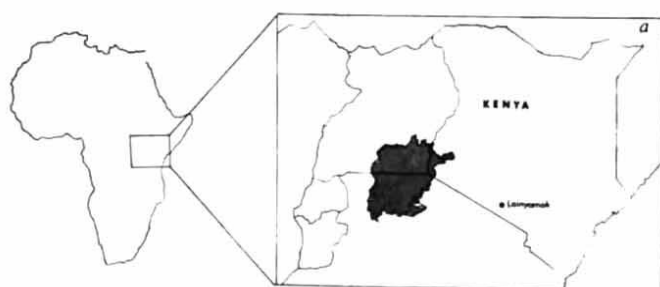


Fig. 1 *a*, Map of Kenya showing the location of the Lainyamok fossil locality. *b*, This Landsat photograph of southern Kenya shows the location of the Lainyamok fossiliferous sediments, Lake Magadi, and the nearby Acheulian site, Olorgesailie. (Photo courtesy of NASA.)



Fig. 2 Occlusal (left) and lingual (right) views of the Lainyamok hominid, a right $P^4 M^{1-2}$ attributed to *Homo* sp. All three teeth are heavily worn; M^2 shows a facet, indicating that the third molar was present in life. The teeth are shown held in anatomical position by matrix; no alveolar bone persists. Note that both P^4 and M^2 are splayed by matrix-filled cracks; the dimensions in Table 2 are corrected for this problem.

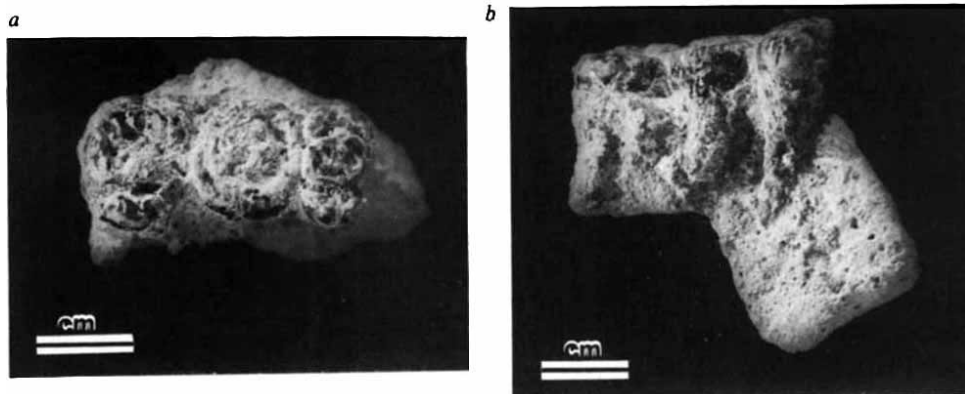
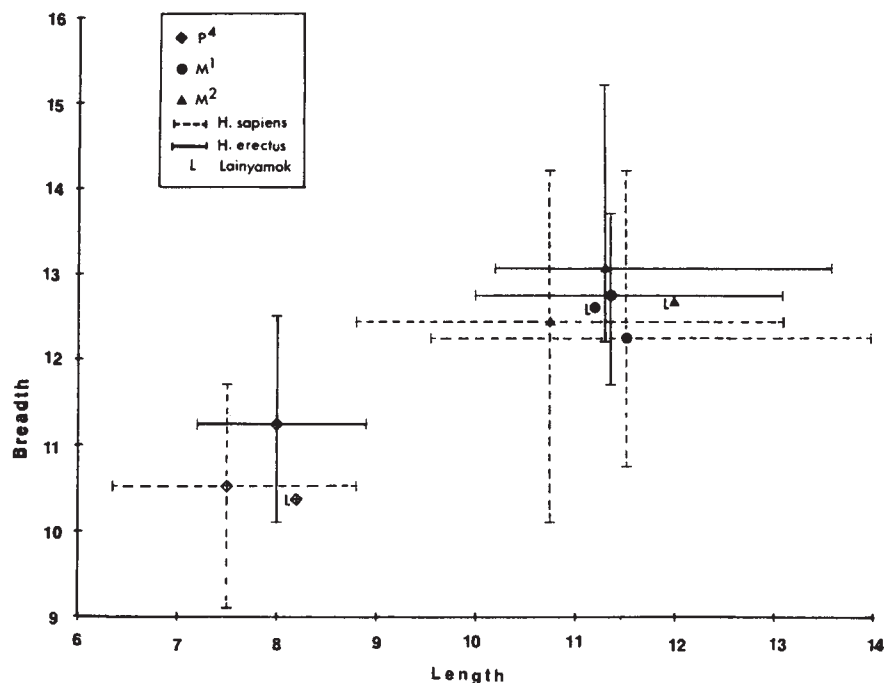


Fig. 3 The dimensions of the Lainyamok teeth are compared with the means and ranges for P^4 M^{1-2} of a sample of *H. erectus* and of a pooled sample of archaic *H. sapiens* including Neanderthals. Neanderthals and other archaic *H. sapiens* are treated as a single sample because Student's *t*-tests reveal no significant differences in the sizes of their cheek teeth. *Erectus* P^4 specimens are significantly broader and longer than those of *sapiens* (Student's *t*-tests: length, $t = 2.067$, $P = 0.022$, d.f. = 37; breadth, $t = 3.2781$, $P = 0.001$, d.f. = 36). *Erectus* molars tend to be broader than those of *sapiens*, but the difference is not significant at the $P < 0.05$ level. *Erectus* second molars are significantly broader than those of the *sapiens* sample (Student's *t*-test: $t = 1.956$, $P = 0.29$, d.f. = 34). Note that the Lainyamok teeth are generally large but consistently fall within the ranges for both samples. (All measurements are in millimetres.) (The *H. erectus* sample included measurements on KNM-ER 3733, Sangiran 4 and 17, Rabat and the original Choukoutien materials from refs 19, 20 and A. Walker (personal communication). The archaic *H. sapiens* and *Homo neanderthalensis* data for specimens from Skhul, Qafzeh, Krapina, Arcy-sur-cure, La Ferrassie, Montsempron, La Quina, Tabun, Kulnal, Amud, Le Moustier and Chatelpéron were taken from ref. 19 and E. Trinkaus (personal communication).)



The Lainyamok sediments are certainly younger than the underlying trachytes (about 0.9–0.7 Myr); if Eugster and Bishop are correct, they are older than about 0.4 Myr.

Beds in the Lainyamok succession suggest alternating lacustrine and subaerial/lake margin conditions. Some beds are well laminated, diatomaceous sediments containing fossil fish and prominent algal limestone horizons. Thus, the Lainyamok graben may have contained part of a fault-bounded lake, possibly Lake Oloronga^{4,10}. Repeated cycles of transgressions—represented by fine-grained, often laminated sediments bearing fossils of terrestrial animals—are interspersed with regressions—as shown by calcrete formation, mild erosion of underlying beds, or mud-cracking. The artefacts and vertebrate fossils described here occur within and on a thick (1.7 m) khaki tuff. This fine-grained, tuffaceous silt or silty-sand, with rootcasts at its top, was waterlain in lake margin or lake flat conditions.

Approximately 200 fossil specimens from the khaki tuff are identified in Table 1. A single hominid individual, adult at the time of death, is known from three teeth (RP^4M^{1-2}) held in anatomical position by matrix (Fig. 2, Table 2). The second molar bears a clear facet for the third molar. Figure 3 compares the Lainyamok dental dimensions with samples of *H. erectus* and archaic *H. sapiens* teeth. The Lainyamok teeth are large mesiodistally, making them more *erectus*-like, but they are within the range of variation of both samples in dimensions and morphology.

Other mammalian species range from small (cane rat, hyrax, hare, murid, spring hare) through an intermediate size (bovid, equid, hyena, suid) to large (giraffid, rhino). Fish, a terrestrial gastropod, ostrich eggshell and a snake are also represented. *Papio* sp. is the only non-hominid primate from Lainyamok to date. Overall, the fauna is consistent with a middle Pleistocene age, but the only extinct genus (identified from horn cores and teeth) is *Parmularius*. Jaws and teeth are numerous (38% of the elements), as are limb (45%) elements. No evidence of hydraulic winnowing^{12,13} is present.

The collected fauna includes 15 partial skeletons; additional articulated or associated partial skeletons remain *in situ*. We know of no other site of comparable age with a similar proportion of articulated or associated material. Bones often occur in clusters 1–2 m in diameter that appear to be single animal scatters¹⁴. Five such clusters also contain cores or flakes;

Table 2 Dimensions of the Lainyamok teeth

Tooth	Length (M-D)	Breadth (B-L)
RP ⁴	8.2	12.8
RM ²	11.2	12.6
RM ²	12.0*	14.4

All measurements in millimetres.

* Estimated value.

M-D, mesiodistal; B-L, buccolingual.



Fig. 4 The stone artefacts from Lainyamok. Top row (left to right): fragment of a lava flake; discoidal hand-axe of green lava; discoidal core of dark-grey lava. Bottom row (left to right): small hand-axe of green lava; quartzite chopper; quartzite spheroid. Some but not all of the artefacts were collected *in situ* from the khaki tuff; others were in the surface lag.

artefacts lie within a few metres of other clusters. Although more rigorous evidence is needed, these data suggest that Lainyamok preserves animal death sites at which hominids may have exploited carcasses.

A thin scatter of isolated artefacts occurs in and on the surface of the khaki tuff; a single cluster of lava flakes may be a manufacturing site. Small, unretouched flakes predominate. Only six artefacts have been collected from Lainyamok to date (Fig. 4); several dozen are visible at the site. The small, well-made hand-axes, unretouched flakes and other core tools are

consistent with an Acheulian industry. Artefacts were made from two local varieties of lava and apparently exotic quartz, quartzite and chert.

Lainyamok can be compared with Olorgesailie, the middle Pleistocene site north-east of Lake Magadi, as both sites sample broadly similar lake margin or lake flat habitats¹⁵. However, Olorgesailie and Lainyamok differ in that: (1) Olorgesailie is rich in giant geladas (*Theropithecus oswaldi*) but lacks *Papio*, the only monkey at Lainyamok; (2) only four carnivore specimens are known from Olorgesailie (C. Koch, personal communication), whereas 21 specimens of at least four carnivore genera are known from the much smaller Lainyamok collection; (3) large hand-axes and cutting or heavy-duty tools typify the Olorgesailie assemblage¹⁵ whereas the known Lainyamok artefacts are small or light-duty implements.

It is not clear if the faunal contrasts are linked to the archaeological differences. Olorgesailie was apparently repeatedly occupied by many hominids engaged in specific, highly organized activities (for example, extensive tool manufacture¹⁵ or systematic butchery of large animals¹⁶). Lainyamok's paucity of

artefacts, single animal scatters and sole artefact cluster suggest it was transiently occupied by only a few hominids. A working hypothesis is that Lainyamok preserves small, short-term occupations where more opportunistic killing and butchering of carcasses of smaller species occurred. Such small-scale sites are common in the ethnographic record^{17,18}, but are not known from the Pleistocene.

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Relative fitness can decrease in evolving asexual populations of *S. cerevisiae*

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It is generally accepted from the darwinian theory of evolution that a progressive increase in population adaptation will occur in populations containing genetic variation in fitness, until a stable equilibrium is reached and/or the additive genetic variation is exhausted. However, the theoretical literature of population genetics documents exceptions where mean population fitness may decrease in response to evolutionary changes in gene frequency, due to varying selective coefficients¹⁻⁴, sexual selection^{5,6} or to epistatic interactions between loci⁷. Until now, no examples of such exceptions have been documented from fitness estimates in either natural or experimental populations. We present here direct evidence that, as a result of epistatic interactions between adaptive mutations, mean population fitness can decrease in asexual evolving populations of the yeast *Saccharomyces cerevisiae*.

Populations of haploid and diploid *S. cerevisiae* were grown in glucose-limited continuous cultures (chemostats) for up to 300 generations. Population sizes were large—approximately 4.9×10^9 for the haploid population and 4.5×10^9 for the diploid population. In these conditions reproduction is exclusively asexual by budding, and evolutionary changes occur by the replacement of one adaptive clone by another. As recombination is virtually absent in these populations, two or more adaptive

mutations must be incorporated into the population in a strict sequential fashion.

Adaptive changes were identified by monitoring the frequencies of independent neutral or weakly selected mutations in the populations. Being asexual, these evolving populations may be regarded as consisting of a series of clones over time, each new clone having a selective advantage over the immediately preceding clone. In each clone the initial frequencies of the independent neutral or weakly selected mutations will be zero, and their frequencies will increase over time at the mutation rate or slightly less, depending on the intensity of selection against the independent marker. The frequencies of the independent mutations will necessarily be lower in the newly emergent adaptive clone than in the preexisting clone, as the former has had less time to accumulate mutations. Therefore, during an adaptive change the population frequencies of the independent mutations will exhibit transient decreases. Repeated replacements of adaptive clones will result in repeated fluctuations in the frequency of the independent markers. These fluctuations, known as periodic selection in the microbiological literature, allow the unambiguous detection of all adaptive changes without prior knowledge of the phenotype of the adaptive mutation itself⁸.

The frequencies of mutants resistant to canavine, cycloheximide and 5-fluorouracil were monitored in the haploid population, and mutants resistant to cycloheximide and 5-fluorouracil were monitored in the diploid population. (Canavanine resistance is recessive and so could not be monitored in the diploid population.) Samples were taken every three to six generations (12-24 h) and aliquots of the cell suspension were stored at -70 °C in 15% glycerol for future analysis. All markers monitored showed the same pattern of fluctuations in each population. From these data, 4 adaptive changes were identified in 245 generations in the haploid population and 6 adaptive changes were identified in 305 generations in the diploid population. The dynamics of the fluctuations of canavanine resistance for the haploid population and cycloheximide resistance for the diploid population are shown in Figs 2 and 3 of ref. 8.

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To determine the epistatic interactions between the adaptive mutations, cells isolated after successive adaptive shifts, containing different numbers of adaptive mutations, were placed in pairwise competition in the same glucose-limited chemostat environment. The fitness of each adaptive clone was estimated

Table 1 Relative fitnesses of the adaptive clones isolated from the haploid *a* population

Strain 1		Strain 2		Fitness of strain 2 relative to fitness of 1 for strain 1 $\pm$ s.e.m. per generation
Isolated at generation	Putative no. of adaptive mutations	Isolated at generation	Putative no. of adaptive mutations	
<i>a</i> 25*	0	97	1	1.10 $\pm$ 0.01
30	0	97*	1	1.11 $\pm$ 0.01
97*	1	120	2	1.05 $\pm$ 0.01
133*	2	203	3	1.16 $\pm$ 0.02
203*	3	245	4	1.10 $\pm$ 0.02
<i>b</i> 30*	0	133	2	1.08 $\pm$ 0.01
30	0	133*	2	1.18 $\pm$ 0.03
30	0	203*	3	0.85 $\pm$ 0.03
30	0	203*	3	0.84 $\pm$ 0.04
30	0	245*	4	1.16 $\pm$ 0.04
30*	0	245	4	1.12 $\pm$ 0.01
97*	1	203	3	1.08 $\pm$ 0.01
102*	1	245	4	1.08 $\pm$ 0.01
133*	2	245	4	1.09 $\pm$ 0.02

The adaptive clones were isolated from long-term glucose-limited continuous cultures inoculated with the haploid *a* strain CP1AB-1A²². Experiments were carried out as described previously⁸. For each experiment the strain which had been isolated after the smaller number of generations of growth in the chemostat is designated as strain 1.

* Strain marked with canavanine resistance. Canavanine resistance has been shown to be neutral in our conditions⁸. Fitnesses ($\pm$ s.e.m.) were calculated as described previously²³ relative to a fitness value of unity for strain 1.

in this way relative to each other adaptive clone. For each experiment one of the two strains was marked with an appropriate resistance marker (canavanine for diploids and 5-fluorouracil for haploids) to allow the two strains to be distinguished. Competition experiments between strains carrying canavanine or 5-fluorouracil resistance and the same strains without the marker, showed canavanine resistance to be neutral and 5-fluorouracil to be selected against⁸ (selective coefficient = 0.09). Therefore, all experiments using the 5-fluorouracil resistance marker were corrected for selection against this marker. To correct for possible interactions between the adaptive mutations and canavanine or 5-fluorouracil resistance, many of the competition experiments were performed twice, with one strain marked with the resistance marker in the first experiment and the other strain marked in the second experiment. No interactions between the resistance markers and the adaptive mutations were observed.

The results for the haploid and diploid populations are shown in Tables 1 and 2. It is important to realize that, since the populations are asexual, each adaptive mutation becomes part of the genetic background in which further adaptive mutations are selected. Therefore, a strain carrying a new adaptive mutation also carries all the adaptive mutations previously fixed in the population. Tables 1*a* and 2*a* show the results of competition experiments between clones differing in only one adaptive mutation. These experiments therefore reconstruct each putative adaptive shift. As expected, the relative fitnesses of the later isolated clones were always higher than those for the clones isolated immediately preceding the adaptive shift. Thus, these results confirm the occurrence of the adaptive shifts in the population signalled by the fluctuations in the frequency of canavanine resistance (haploids) or cycloheximide resistance (diploids).

Tables 1*b* and 2*b* show the results of competition experiments between clones differing by more than one adaptive mutation. These data clearly reveal significant epistasis between the adaptive mutations. In all cases the fitness differentials between adaptive clones differing in more than one adaptive mutation

Table 2 Relative fitnesses of the adaptive clones isolated from the diploid *a/a* population

Strain 1		Strain 2		Fitness of strain 2 relative to strain 1 $\pm$ s.e.m. per generation	Relative fitness of strain 2 corrected for selection of fluorouracil resistance marker
Isolated at generation	Putative no. of adaptive mutations	Isolated at generation	Putative no. of adaptive mutations		
<i>a</i> 26*	0	101	1	1.41 $\pm$ 0.03	1.25
26	0	101*	1	1.06 $\pm$ 0.02	1.16
101*	1	139	2	1.18 $\pm$ 0.04	1.06
101	1	139*	2	0.95 $\pm$ 0.06	1.04
139	2	213*	3	1.01 $\pm$ 0.03	1.10
213*	3	243	4	1.20 $\pm$ 0.02	1.09
213	3	243*	4	1.06 $\pm$ 0.03	1.16
243	4	268*	5	0.96 $\pm$ 0.03	1.05
262*	5	294	6	1.14 $\pm$ 0.04	1.03
<i>b</i> 26	0	139*	2	0.99 $\pm$ 0.01	1.08
26*	0	213	3	1.28 $\pm$ 0.02	1.15
26	0	243*	4	0.88 $\pm$ 0.02	0.97
20	0	262*	5	0.98 $\pm$ 0.08	1.07
26*	0	301	6	0.84 $\pm$ 0.05	0.75
26	0	305*	6	0.71 $\pm$ 0.06	0.80
101*	1	262	5	1.47 $\pm$ 0.10	1.30
101	1	262*	5	1.04 $\pm$ 0.05	1.14
101	1	305*	6	0.95 $\pm$ 0.04	1.04
139*	2	243	4	1.06 $\pm$ 0.10	0.97
139	2	262*	5	0.81 $\pm$ 0.03	0.90
213*	3	268	5	1.16 $\pm$ 0.02	1.05
213	3	268*	5	0.94 $\pm$ 0.01	1.03

The adaptive clones were isolated from long-term glucose-limited continuous cultures inoculated with the diploid *a/a* strain CP1AB²². Experiments were carried out as described previously⁸. For each experiment the strain which had been isolated after the smaller number of generations of growth in the chemostat is designated as strain 1.

* Strain marked with 5-fluorouracil resistance. 5-Fluorouracil is selected against in our conditions (selective coefficient = 0.09). Relative fitnesses ($\pm$ s.e.m.) were calculated as before²³. Fitnesses of strain 2 were corrected for selection against the 5-fluorouracil resistance marker by assuming selective effects to be additive before normalizing the fitnesses relative to a fitness value of unity for strain 1.

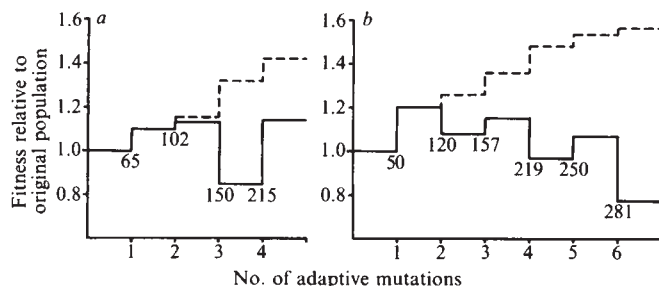


Fig. 1 Change in population fitness relative to a fitness value of unity for the original population of the haploid *a* strain CPIAB-1A (a) and of the diploid *a/α* strain CPIAB-1AB (b). ----, Expected rate of increase of population fitness due to substitution of four (a) or six (b) adaptive mutations, assuming fitnesses are additive. —, Observed rate of change of population fitness due to substitution of four (a) or six (b) adaptive mutations. Standard errors are given in Tables 1 and 2. Fitness values for each adaptive clone are estimated from reconstruction experiments with the original clone used to initiate the experiment. The numbers shown against each change in population fitness are the times of occurrence (generations) for each adaptive mutation, estimated as described previously⁸.

are smaller than those expected from the sum of the fitness differentials between clones differing in only one adaptive mutation. For example, in Table 1 the fitness differential between the first adaptive clone carrying 1 adaptive mutation and the clone isolated at generation 245 carrying 4 adaptive mutations is only 0.08. The predicted differential assuming no epistasis, calculated from Table 1a, is $0.05 + 0.16 + 0.10 = 0.31$. Of particular interest, however, is the nature of certain of the epistatic interactions. In one case in the haploid population, and in several cases in the diploid population, the relative fitnesses of adaptive clones carrying multiple adaptive mutations were lower than the fitnesses of clones isolated earlier in the experiment, even though each clone carrying a new adaptive mutation has an increased fitness relative to the immediately preceding clone. Thus, in both the haploid and diploid populations, the fixation of several adaptive mutations can actually decrease the fitness of a strain relative to the fitness of the clones at the beginning of the experiment. To emphasize this point, Fig. 1 shows the changes in relative fitness of the haploid (Fig. 1a) and diploid (Fig. 1b) populations with respect to the fitness of the original strain as each adaptive mutation becomes fixed in the population. The dashed line in the figure indicates the expected rate of increase of population fitness assuming the adaptive mutations are additive. The expected rate of increase of population fitness, assuming fitnesses are multiplicative, is slightly higher.

This apparent paradox is resolved when it is considered that fitness is a relative measure and is defined by the numbers of descendants left by a given genotype relative to others in the population⁹. The mean fitness of a genotype may depend on many variables each of which may affect the intrinsic rate of natural increase of the population (*r*-selection) and/or the ability of a genotype to alter the numbers of offspring left by its neighbours (*K*-selection)^{4,9}. It is clear that in our experimental system the variable responsible for each adaptive shift may be different. In this context it is not unreasonable that a combination of adaptive mutants may result in a maladaptive clone when compared in competition with a clone isolated several steps earlier. If the adaptive shifts only resulted in a change in the intrinsic rate of natural increase, such decreases in relative fitness should not occur. However, growth in chemostats is always resource-limited and the rate of growth of the population is fixed by the dilution rate¹⁰. Therefore, it is unlikely that the majority of adaptive clones involve change in the intrinsic rate of natural increase.

The suggestion that extensive interactions occur between loci affecting fitness is not new. Such interactions have been observed for loci determining drug resistance in both *Escherichia coli*¹¹

and the bacteriophage T₄ (ref. 12). Furthermore, ample indirect evidence exists from observations of multilocus gametic associations in sexual populations¹³, from the well-known and widespread lack of concordance between predicted and observed responses to selection for components of fitness in animal and plant breeding, and from the results of artificial selection experiments^{14,15}. However, the present results present the first direct evidence for and measurements of epistatic interactions between multiple adaptive mutations in evolving populations. Furthermore, the results represent the first observation that population fitness may decrease in response to evolutionary changes. Such a phenomenon has been postulated theoretically by several workers^{1-4,6,7}.

Our capacity to observe such decreases is clearly dependent on the asexual nature of our populations, in which adaptive mutations occur and are fixed in the population strictly sequentially. In such populations only certain combinations of adaptive mutations will necessarily occur. In sexual populations, recombination potentially will allow the generation of all combinations of adaptive mutations and also will allow the incorporation of mutations in parallel. This may be sufficient to obscure any significant fitness decreases. However, the importance of recombination in sexual populations will be minimized if population sizes are small¹⁶ and if adaptive changes occur sufficiently rapidly that each adaptive mutation is fixed before the occurrence of the next¹⁷. The results described here and previously⁸ suggest that such rates of evolutionary change are not unreasonable. Therefore, sexual populations may also exhibit fitness decreases during evolutionary change.

The rates of occurrence of the adaptive mutations in these and other similar populations⁸ illustrate a further unexpected characteristic of these changes. Figure 1 shows the estimated times of occurrence of each adaptive mutation. The rate of occurrence of adaptive mutations being fixed in the populations did not decrease over time as would be expected if the possible range of adaptive changes for this environment were becoming exhausted. This result may not be so surprising given that the relative fitnesses of the populations are not constantly increasing. Mutations selected for during one period of adaptation may be selected against during subsequent periods of adaptation in a different genetic background. It is possible that certain categories of adaptive mutations are continually being fixed and then lost from the population as their fitnesses become modulated by the genetic background. Thus, for these populations evolution may be 'circular', an infinitely repeating succession of adaptive changes involving a limited repertoire of adaptive mutations^{18,19}. Such a phenomenon has previously been suggested to occur in evolving populations of RNA viruses²⁰. The concept that there is no 'adaptive peak'²¹ corresponding to a single genotype or genetic composition of the population has profound implications for our understanding of the maintenance of genetic variation under directional selection.

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Single neurones can initiate synchronized population discharge in the hippocampus

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The synchronized firing of neuronal populations is frequently observed in the mammalian central nervous system. The generation of motor activities such as locomotion¹ and respiration² requires the simultaneous activation of many neurones and synchronous firing also underlies the cortical α rhythm³ and the hippocampal θ rhythm⁴. However the influence that single neurones may have on such neuronal population discharges is not clear. We have examined this question using small isolated segments of the CA3 region of the guinea pig hippocampus. We report here that in the presence of picrotoxin, a γ -aminobutyric acid (GABA) antagonist, these segments spontaneously generate synchronized rhythmic bursts comparable with the interictal epileptiform discharges observed in the hippocampus and neocortex in the presence of penicillin^{5–7}. The activation of some individual neurones by intracellular current injection can partially entrain and reset the rhythm. The probability that a synchronized burst will follow stimulation of a single cell increases with time after a spontaneous synchronized discharge, suggesting that each population discharge is followed by a period of relative population refractoriness. A delay of 40–200 ms elapses between the activation of a single neurone and the synchronized discharge. We suggest that during this time activity elicited in one neurone spreads to other neurones through multisynaptic excitatory pathways and leads eventually to the participation of the whole population in a synchronous burst.

Transverse slices (400 μ m thick) of guinea pig hippocampus were prepared with a vibratome (Oxford). Two further cuts made orthogonally to the cell-body layer in the CA3 field and meeting at the hippocampal fissure resulted in a segment of neural tissue measuring 400–600 μ m along the stratum pyramidale. Consideration of soma diameter (20–40 μ m) and packing density (two to three cells deep) in the CA3 stratum pyramidale⁸ suggests that segments contained approximately 1,000 pyramidal cells. Segments were transferred to a recording chamber and maintained as described previously⁹. Intracellular and extracellular field potential recordings were made from the stratum pyramidale with 2 M K-acetate-filled microelectrodes of resistances 80–120 M Ω and 40–60 M Ω respectively. Synchronized discharge was induced by exposure to picrotoxin (10^{-4} M) which blocked spontaneous and evoked inhibitory synaptic potentials.

The active and passive properties of more than 70 neurones recorded in this study were similar to those described for pyramidal cells from the same area of the intact hippocampal slice^{10,11}. In the presence of picrotoxin, spontaneous synchronized bursts of period 7.3 ± 2.9 s (mean $\pm$ s.d.) were observed in 27 CA3 segments measuring 400–600 μ m along the stratum pyramidale whereas such activity was never seen in smaller segments. Rhythmic population bursts, when all neurones discharged synchronously with the field potential burst, were blocked by low- Ca^{2+} (0.5 mM) high- Mg^{2+} (8 mM)

solution in six segments. This behaviour therefore seems to require a minimum neuronal substrate for its expression and depends on local synaptic mechanisms within the CA3 region. With the size of neuronal population reduced to the minimum which supports synchronized discharge we tested the capacity of single members of the population to influence population activity. In other neuronal systems this question has been investigated by applying repetitive intracellular stimulation at frequencies differing from that of the population rhythm¹². We found that 10 out of 36 neurones tested could influence the rhythm of synchronized activity (Fig. 1). Repetitive intracellular stimulation partially entrained the population discharge when applied at frequencies 30–60% faster than the mean spontaneous frequency (at higher frequencies synchronized events followed some multiple of the stimulus period as in Fig. 3). The frequency of population bursts increased by $23 \pm 11\%$ of control ($n = 10$) and they often followed an intracellular stimulus with latency up to 200 ms (Fig. 1c). A similar delay may be observed *in vivo* between afferent stimulation and the occurrence of an interictal discharge^{5–7}. The degree of entrainment, expressed as the proportion of synchronized bursts which occurred within 200 ms of intracellular stimulation, varied between 17 and 60% whereas the proportion of bursts expected to occur within this time period by chance was 3–7%.

The influence of a single neurone on population activity was characterized more systematically ($n = 11$) by making single intracellular current injections at different times following a spontaneous synchronized burst (Fig. 2). Even at times much shorter than the mean period of spontaneous bursting the activation of some neurones could elicit a synchronized burst and reset the population rhythm such that the time to the next spontaneous burst was comparable with the mean period of the population rhythm. However the proportion of trials in which a constant intracellular current injection led to a population burst increased with time following a spontaneous burst (Fig. 2c). Failure to elicit a population burst was not due to failure to activate the neurone but instead appears to reflect a period of relative population refractoriness^{5,7,13}, which follows each synchronized burst and which outlasts the intracellular afterhyperpolarization (Fig. 2b). The latency with which a synchronized discharge followed activation of this neurone varied between 40 and 120 ms (Fig. 2d). Synaptic interactions have often been implicated in the synchronization of neuronal activity^{5,14,15}. If synaptic transmission is involved it must be primarily chemically mediated and excitatory since the synchronized activity discussed here was blocked in low- Ca^{2+} high- Mg^{2+} medium and observed in the absence of GABA-dependent inhibition. Anatomical^{16,17} and physiological¹⁸ evidence suggests that pyramidal cells in the CA3 region of the hippocampus possess recurrent excitatory collaterals. However the time elapsing between activation of a single neurone and a following population discharge greatly exceeds that needed for transmission across a single synapse and the short intercellular distances in a segment preclude long axonal impulse conduction times. Therefore, as has been suggested in a theoretical model¹⁹, a synchronized discharge may depend on activity spreading from the activated cell, through local recurrent pathways, to other cells in several steps, with an increasing number of cells discharging at each step until the whole population becomes synchronously active.

Paired intracellular recordings ($n = 10$) were made to examine directly the involvement of synaptic mechanisms in the initiation of a synchronized burst. A multisynaptic connection existed between the two cells shown in Fig. 3. Moreover, activation of the presynaptic neurone could elicit a simultaneous long-latency discharge in both neurones, whereas activation of the postsynaptic neurone had no effect. On stimulating intracellularly at a faster frequency than simultaneous bursts could be evoked, it was apparent that the period of population refractoriness was associated with a reduced postsynaptic response to activation of the presynaptic cell. Here presynaptic current injections of constant amplitude and duration were made at 1-s intervals

Fig. 1 Repetitive intracellular stimulation of a single neurone partially entrains synchronized population activity. To test for entrainment, maintained hyperpolarizing current was injected intracellularly so that the only neuronal activity occurring was that synchronous with the population burst. The mean control interval between population bursts, in this case 6.23 ± 2.20 s (mean $\pm$ s.d., $n = 36$), was established. Repetitive intracellular stimulation was then applied with intensity sufficient to elicit a burst of action potentials and with interval shorter than control, here once every 4 s. Traces in *a*, *b* and *c* are from above-intracellular potential and current injected through the recording microelectrode. *a*, Intracellular stimulation began approximately halfway through the record. Although the first two stimuli had no effect, synchronized population bursts at higher frequency than before stimulation closely followed the intracellular bursts elicited by the next three stimuli shown. *b*, Cellular activation was followed closely by a population burst on six out of eight occasions showing that entrainment was not complete. *c*, Current injection elicited a burst of action potentials with no field potential correlate followed, with a latency of 90 ms, by a synchronized population burst reflected in both intracellular and field potential records. During this period of repetitive intracellular stimulation 22 out of 36 population bursts followed a stimulus with latency of less than 200 ms. If stimulation has no effect on the population rhythm, two population bursts would have been expected to occur with this latency. *d*, Distribution of intervals between population bursts preceding (control) and during (stimulus) repetitive stimulation. The mean control interval was 6.23 ± 2.20 s (mean $\pm$ s.d., $n = 36$). During stimulation once every 4 s, the mean interval was 4.35 ± 1.10 s ($n = 36$), significantly ($P < 0.01$) lower than control.

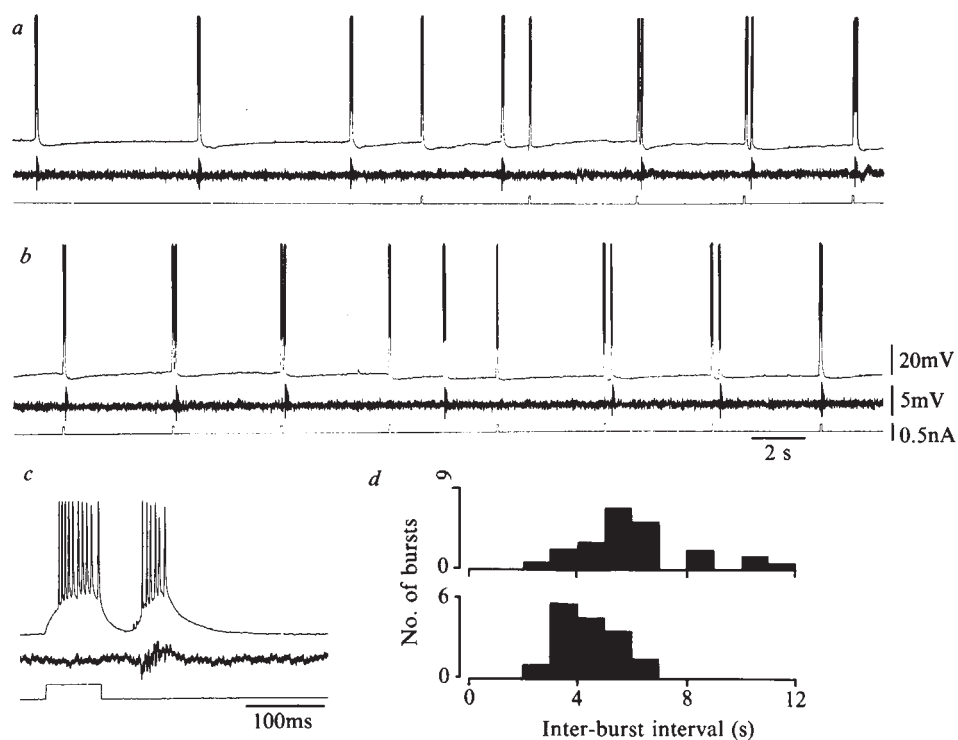


Fig. 2 Variation of the influence of a single neurone on population activity with time following a spontaneous synchronized burst. The mean interval between spontaneous population bursts in this segment was 6.3 ± 1.9 s (mean $\pm$ s.d., $n = 40$). The effect of intracellular current injection, of constant amplitude and duration, was tested at times ranging from 1 to 6 s after a spontaneous synchronized burst, with at least five cycles of spontaneous activity allowed to occur between each test. *a*, Each test shown at times from 1 to 6 s after a spontaneous synchronized burst, except that at 1 s, successfully elicited a synchronized population burst. When the population rhythm was reset, the mean time to the following spontaneous population burst was 7.1 ± 2.3 s (mean $\pm$ s.d., $n = 27$). The traces are from above-intracellular potential, extracellular field potential and injected current. *b*, Spontaneous activity (membrane potential and field potential) with interval between synchronized bursts close to its mean value. *c*, *d* (Plotted with the same time scale as *b*) show respectively the per cent success of the same intracellular stimulus in eliciting a synchronized burst and the latency with which a burst followed neuronal activation for current injections made at different times after a spontaneous population burst. Seven to 13 tests of the effect of current injection were made at each second from 1 to 6 s after a spontaneous synchronized burst.

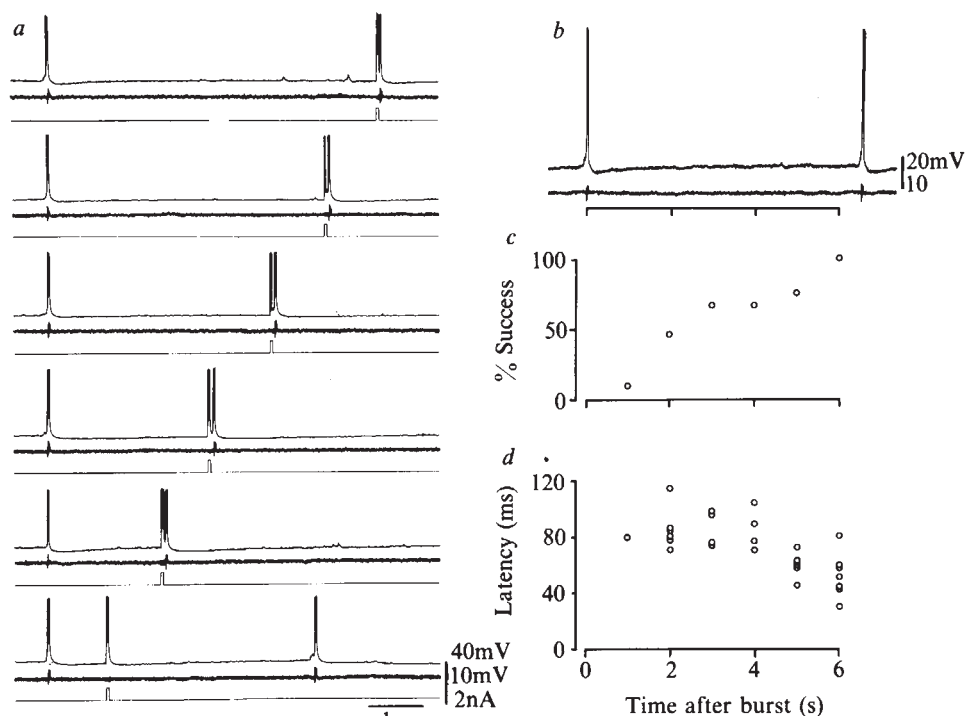
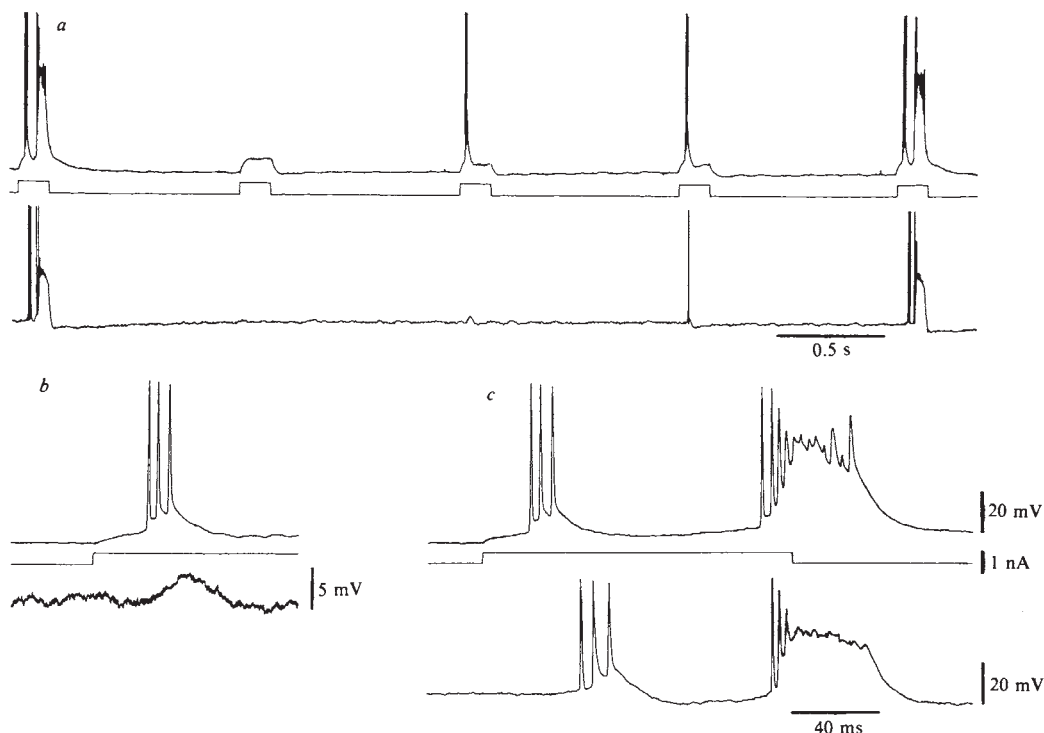


Fig. 3 Simultaneous intracellular recordings from a neurone which influenced synchronized activity (upper trace) and a neurone to which it was synaptically connected (lower trace). Current pulses of constant amplitude and duration (middle trace) were injected into the presynaptic cell at a frequency of 1 Hz. *a*, The first and fifth current injections shown elicited action potentials in both cells followed by a spontaneous long-latency burst. *b*, Expanded traces of the third stimulus. Presynaptic activation elicited a postsynaptic potential of amplitude 3.2 mV and latency from presynaptic activation of 7.8 ms. The connection was judged to be a chemical synapse since hyperpolarizing current pulses injected into the presynaptic cell caused no postsynaptic voltage change. The connection was not monosynaptic since the latency of the synaptic potential was long and variable: the mean latency was 13.3 ± 5.9 ms and mean amplitude was 2.3 ± 0.7 mV ($n = 33$). *c*, Activation of the presynaptic neurone elicited a burst in the postsynaptic neurone followed by a synchronous burst, similar to the intracellular correlate of a population burst, in both neurones. The onset of activity in these two cells during a synchronous burst was not exactly simultaneous, varying by up to 7 ms with neither cell consistently discharging before the other.



following a simultaneous burst (Fig. 3*a*). In the first instance the presynaptic neurone was not activated, at 2 s a presynaptic burst evoked a synaptic potential but not an action potential in the other cell (Fig. 3*b*), and at 3 s a presynaptic burst elicited a single postsynaptic action potential. When a simultaneous burst was elicited (Fig. 3*c*) a presynaptic burst evoked a postsynaptic burst with a latency of 14–33 ms and the simultaneous burst followed with a latency of 55–120 ms. Although this is a single instance, two properties of local excitatory synapses noted by McVicar and Dudek¹⁸ and ourselves (unpublished observations) in paired intracellular recordings from the CA3 region of the intact slice may be important in the generation of synchronized activity. First, postsynaptic potentials are large, compared with those observed in spinal motoneurons²⁰ for instance, so that activity in one neurone may result in the discharge of a postsynaptic neurone to which it is coupled. Second, local recurrent excitatory contacts appear to be rare, being found in only about 5% of paired recordings in the intact slice. This paucity of recurrent connections may account for the long latencies with which synchronized bursts are elicited.

The findings reported here show that some single neurones can influence the synchronized discharge of a mammalian neuronal population. We suggest that this influence depends on transmission through multisynaptic excitatory pathways. This form of population behaviour, which occurs in the CA3 region of the hippocampus, is therefore different from the synchronized discharge observed in the CA1 region which does not depend on chemical synaptic transmission^{21,22}. The powerful neuronal signal amplification described here, where activity induced in one cell influenced the behaviour of a much larger neuronal population, was demonstrated in the absence of GABA-dependent inhibition. Such mechanisms may also operate to some

extent in more physiological conditions, since it appears that inhibition mediated by GABA may be particularly labile²³. Finally, we have found that only a proportion of neurones can influence population activity. It remains to be determined whether these cells possess unusually widely arborizing local axon collaterals or whether the synaptic contacts which they make are particularly powerful.

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Identification of proopiomelanocortin neurones in rat hypothalamus by *in situ* cDNA-mRNA hybridization

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Adrenocorticotrophic hormone (ACTH), β -endorphin and the melanotropins (MSHs) are all derived from a single large precursor molecule, proopiomelanocortin (POMC)¹⁻⁵ by individual processing through a series of co- and post-translational modifications. Although the primary site of synthesis is in the pituitary, POMC-derived peptides have been identified in various tissues, notably the brain (see refs 6, 7 for review). A major question concerning brain POMC is whether it is synthesized within the central nervous system (CNS) itself or whether it is taken up from plasma flowing in a retrograde fashion from the pituitary. POMC peptides have been detected immunohistochemically and biochemically in the medial basal hypothalamus, the amygdala and throughout the brain stem. POMC peptide-containing cell bodies have been identified only in two cell groups, however, principally in the periaruate region of the hypothalamus and to a lesser extent in the nucleus of the tractus solitarius⁸⁻¹⁰. These and other^{11,12} observations have suggested that POMC peptides are synthesized locally in the medial basal hypothalamus and reach other regions of the CNS by axonal transport. Civelli *et al.* identified POMC mRNAs in nucleic acid extracts of rat and bovine hypothalami by solution hybridization as well as Northern gel blot analysis¹², but because of the close proximity of the hypothalamus to the pituitary and the extremely low amounts of POMC mRNA being measured in the hypothalamus, the possibility of tissue contamination during dissection could not be ruled out. We report here the anatomical co-localization of POMC-related peptides and POMC-specific mRNAs to a single major cell group in the medial basal hypothalamus. The presence of POMC-specific mRNA in a POMC peptide-containing cell in the brain is strong support for POMC biosynthesis within brain tissue.

To localize the POMC-specific mRNA in single cells, we used the refined technique of *in situ* cDNA-mRNA hybridization on frozen 10- μ m rat brain sections. This technique is conceptually very similar to immunohistochemistry, except that a DNA or RNA probe is used to detect the presence of a given nucleic acid sequence¹³⁻¹⁵. In this study, a cDNA probe is used to identify any nucleic acid sequences complementary to the POMC gene, principally POMC mRNA and heteronuclear RNA (hnRNA) in the cytoplasm and nucleus of cells. Tissues were fixed by perfusing the animal, through the aorta, with 4% paraformaldehyde in phosphate-buffered saline (PBS) for 20 min. This fixation method preserves both the peptides and mRNAs in the cytoplasm of cells for subsequent analysis. The fixed brain was partitioned into three blocks by two coronal cuts, one rostral and one caudal to the hypothalamus. The blocks of tissue were placed in 15% sucrose in PBS at 4 °C overnight to rinse out excess fixatives, and then embedded in OCT compound (a water-soluble supporting medium from Tissue Tek) and frozen in liquid nitrogen. Ten-micrometre sections were cut

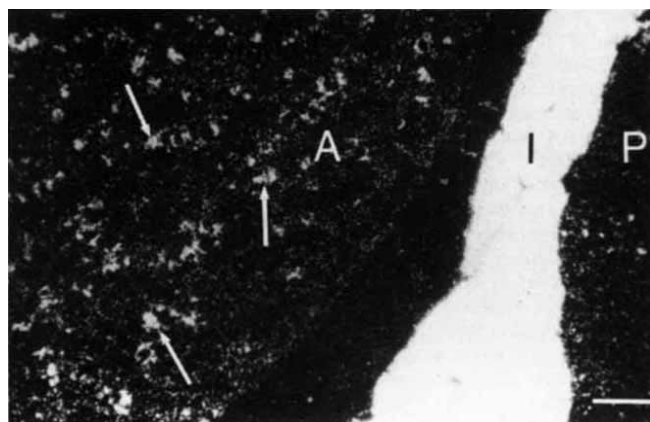


Fig. 1 *In situ* cDNA-mRNA hybridization of POMC-producing cells of the anterior and intermediate lobes of rat pituitary. The anterior lobe (A) corticotrophs can be identified by the dense patches of autoradiographic grains (arrows indicate three of the many such cells). The intermediate-lobe (I) melanotrophs all contain POMC mRNA and can be seen as a very dense streak of white autoradiographic grains. The posterior lobe (P) shows background staining. The I-13 rat POMC cDNA clone was constructed from a poly(A)⁺ RNA preparation obtained from neurointermediate lobe, by reverse transcription and (dG-dC)_n tailing^{19,20}. The resulting 550-base pair probe (I-13) was isolated, sequenced and shown to code for the amino acid sequences of POMC from the middle of the NH₂-terminal glycopeptide to the carboxy terminus as well as a segment of the 3'-untranslated region not including the poly(A) tail. The I-13 sequence is identical to that published by Drouin and Goodman³. The I-13 insert was restriction-cut from the pBR322 plasmid and made radioactive by nick-translation²¹, using all four tritiated triphosphates, to a specific activity of 2–5 × 10⁶ c.p.m. per μ g DNA. After perfusing the rat with 4% neutral buffered formalin, the tissue was removed, kept in 15% sucrose overnight, immersed in OCT mounting medium, frozen and sectioned. Tissue sections were thawed at 25 °C and rinsed in diethyl pyrocarbonate (DEP)-treated water (50 μ l DEP per 250 ml water). The radioactive I-13 POMC probe was heat-denatured for 5 min at 100 °C in hybridization buffer¹⁵ and immediately cooled in ice water. 2–5 × 10⁴ c.p.m. of labelled probe in 5 μ l of hybridization buffer was pipetted onto the section and reacted for 18 h at 28 °C. The section was washed in 2×SSC at room temperature for 4–5 h, changing wash every 15 min. The washed sections were then dehydrated through graded alcohols and air-dried. They were then dipped in Ilford L-4 nuclear emulsion (Polysciences, Pennsylvania), liquified 1:2 with water, air-dried for 1 h in the dark and exposed (10 days) in light-proofed boxes, with desiccant. Development was carried out in Kodak D-19 (diluted 1:1 in water) for 2–4 min at room temperature and fixed in Kodak rapid-fix for 2–3 min, and washed in water for 20 min. Scale bar, 100 μ m.

with a cryostat microtome onto gelatin-coated glass microscope slides. The hybridization and autoradiographic procedure was as described previously¹⁵, using a tritium-labelled nick-translated POMC cDNA.

Validation of the *in situ* method and the ³H-POMC cDNA probe is shown for rat pituitary (a tissue known to synthesize POMC) in Fig. 1. Silver grains are localized over the cytoplasm of cells in both the intermediate and anterior lobes of the pituitary, paralleling the distribution previously established by immunohistochemistry for POMC cells. Serial sections staining with an anti-ACTH antibody have confirmed these cells to be POMC peptide-producing cells. There is no labelling of other cells in the tissue section. The signals have been shown to represent specific POMC cDNA-mRNA hybrids¹⁵.

Using identical procedures, coronal brain sections were hybridized with tritium-labelled POMC cDNA. Figure 2 shows the cytoplasmic localization of silver grains in specific cells in the medial basal hypothalamic region. Similar distributions of silver grains were seen in sections rostral and caudal to the section in Fig. 2, paralleling the known distribution of POMC

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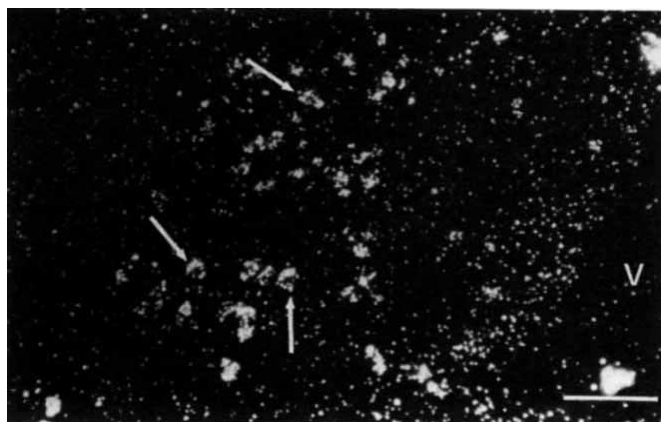


Fig. 2 *In situ* cDNA-mRNA hybridization of the POMC cells in the periaruate region of medial basal hypothalamus. Autoradiographic exposure was for 5 weeks. Arrows indicate three of the several autoradiographically marked POMC cells. The third ventricle is seen at the right side of the photograph (V). The method is as described in Fig. 1 legend. Scale bar, 100 μ m.

neurones in the hypothalamus. There was no similar cytoplasmic localization of POMC mRNA anywhere else in the brain. Treatment of the tissue sections with 25 μ g ml⁻¹ pancreatic ribonuclease A at 42 °C for 1 h, before hybridization with POMC cDNA, gave no autoradiographic signal, showing that the positive signal obtained was derived from cDNA-mRNA hybrids (data not shown). When we hybridized brain sections with either nick-translated rat growth hormone cDNA or plasmid pBR322, there was no detectable localization above background in other cells or cells in the periaruate region of the rat brain (for

example, see Fig. 2). These observations show that our method is specifically identifying POMC mRNA in individual hypothalamic cells.

To characterize further the cytoplasmic localization, serial sections on either side of those labelled with ³H-POMC-cDNA probes were stained with anti-ACTH antibody. Figure 3 shows three serial sections of the rat medial basal hypothalamic region, stained with anti-ACTH sera (a, c) or hybridized *in situ* with a ³H-labelled POMC-specific DNA probe (b). Many of the cells in the periaruate region were labelled with both POMC cDNA and anti-POMC antibodies, thus providing evidence for POMC biosynthesis in brain (that is, localizing POMC mRNAs and its corresponding peptides in the same neurone). A few cells showed labelling for only POMC mRNAs or peptides. In such serial section analysis, some cells stain with antisera alone and others react with cDNA probes alone. Often a cell is present in one 10- μ m section but not present in the next. Only data in which the same cell could be clearly identified in two (or more) serial sections were interpreted. In those conditions we can conclude that most (if not all) POMC cells in the arcuate region are marked by both the antisera and the cDNA probe.

We have used the *in situ* hybridization technique to localize a specific mRNA within individual cells in the mammalian brain. Although the presence of a given mRNA in a cell does not necessarily mean that the mRNA is being actively translated, the concomitant presence of its corresponding protein product in the same cell is *prima facie* evidence for the endogenous biosynthesis of that peptide. Thus, the co-localization of the POMC mRNA by *in situ* cDNA-mRNA hybridization and the POMC protein products by immunocytochemistry within the same cells in the arcuate region of the rat hypothalamus provides the strongest evidence so far for the actual site of synthesis of a mammalian neuropeptide.

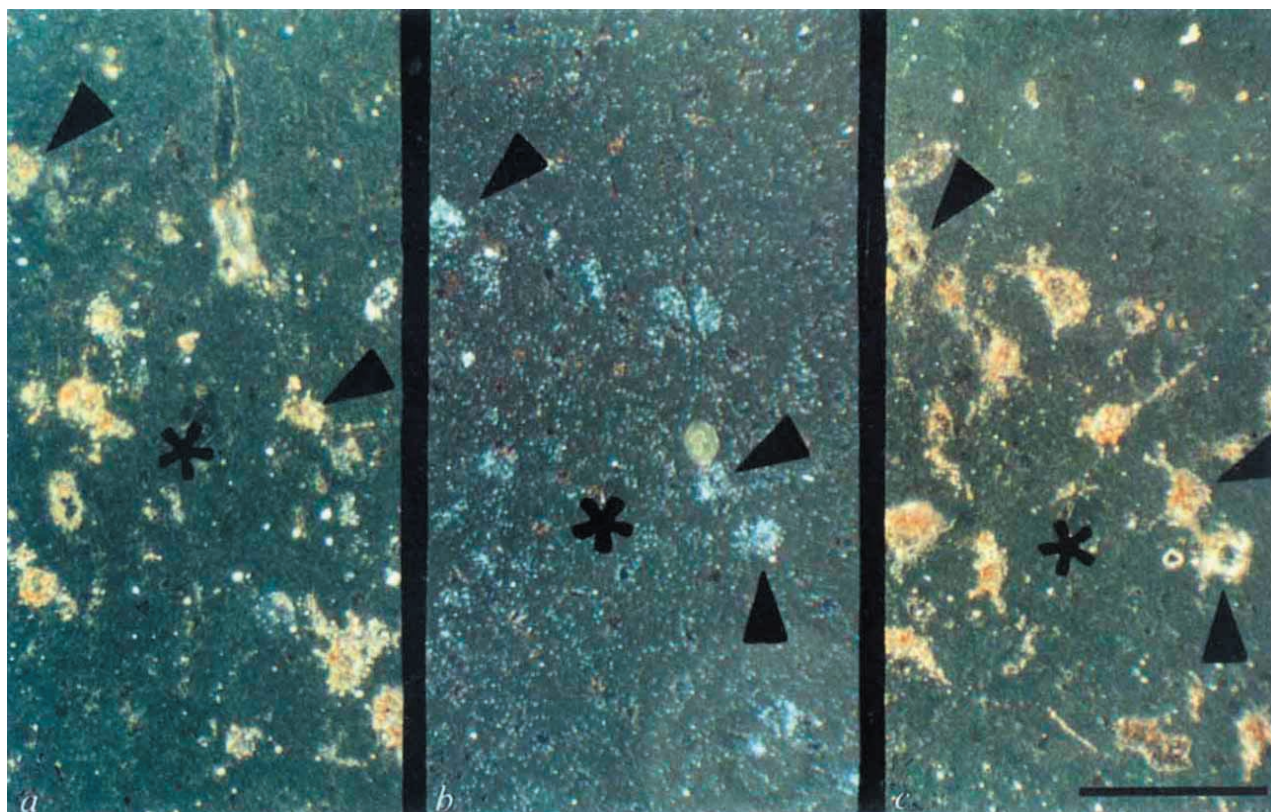


Fig. 3 Co-localization of ACTH immunoreactivity and POMC mRNA in the same cells of rat hypothalamus. *In situ* cDNA-mRNA hybridization of brain POMC neurones (b) was carried out as described for Fig. 1 using the POMC-cDNA 1-13 probe. In 10- μ m serial sections, immunocytochemistry of brain POMC neurones was carried out using the peroxidase-antiperoxidase method and antibody directed against ACTH (a, c). In unpublished studies this serum has been shown to be blocked by ACTH(1-39) and -(1-24), but not by α -MSH, β -endorphin or other known peptides. Arrows indicate several cells which both stained with anti-ACTH immunoperoxidase reaction and hybridized to the ³H-labelled 1-13 probe. Asterisks lie over an artery common to all three sections. Note the tight distribution of autoradiographic grains over the POMC cells. Scale bar, 100 μ m.

Civelli *et al.* have found POMC-like mRNA in other regions of the brain¹². There are several possible reasons why we did not find any *in situ* cell-body localization outside the hypothalamus. One is that the *in situ* cDNA-mRNA hybridization method we used was not sensitive enough to detect a POMC mRNA level much below that found in the hypothalamus. The data reported by Civelli *et al.* indicated that there is 10 times less POMC-like mRNA in the rat amygdala and cerebral cortex relative to the hypothalamus¹². On the other hand, if the POMC mRNA in the amygdala was present at the same level per cell as that of the hypothalamus, and therefore in fewer cells, there would be a total of only 50–100 POMC cells in the whole amygdala. This small number of cells spread over such a large brain region may not have been detectable by our analysis. Furthermore, immunocytochemical studies using a variety of POMC-cross-reacting antisera have not been able to demonstrate any POMC cell bodies in either the amygdala or cortex, which is in agreement with our *in situ* mRNA studies. An alternative explanation may be that the POMC-like mRNA found outside the hypothalamus is not the authentic POMC mRNA. It may consist of a collection of cross-reacting species of mRNAs present in a large number of cells. If this were the case, the signal would be too dilute to be detectable over background in an *in situ* cDNA-mRNA hybridization experiment.

Since the *in situ* cDNA-mRNA hybridization technique can detect a specific mRNA within a single cell, when optimized, it will be especially useful in the study of the regulation of gene expression in complex systems such as the CNS. For example, there are about 10,000 POMC neurones in the periaruate region of the hypothalamus, and they may not all respond identically to the same hormone or neurotransmitter modulators. Indeed, only a small percentage of the periaruate POMC neurones can concentrate oestrogen¹⁶, which is known to modulate the distribution and content of hypothalamic POMC peptides. Experiments measuring rat hypothalamic POMC mRNA levels during various endocrine states including ovariectomy have been done in this laboratory. Total RNAs were extracted from whole hypothalami, covalently bound to nitrocellulose paper and hybridized to POMC-specific cDNA probes. No reproducible variation of more than 20% from the control level was found. This may be explained by the differential regulation of the POMC gene in these POMC neurones. Since only a small percentage, about 10%, of these neurones concentrate oestrogen, that is, have primary responsiveness towards the steroid, then even if oestrogen has a dramatic effect on POMC gene expression in these cells it may be masked by the pool of POMC mRNA contributed by non-responsive neurones. This type of difficulty was encountered by Nakanishi *et al.* when they studied the glucocorticoid regulation of pituitary POMC gene expression⁵. Glucocorticoids suppress strongly the anterior-lobe POMC mRNA level but leave the intermediate-lobe POMC mRNA level apparently unaffected. Fortunately, the two lobes of the pituitary gland can be readily dissected, so that subsequent studies were able to show a more exact pattern of regulation of the expression of the POMC gene^{17,18}. However, to dissect out neurones which are regulated differently would be a formidable task. An alternative method would be to look at these neurones individually or in endocrine-responsive subgroups by *in situ* cDNA-mRNA hybridization, so that one may obtain a more accurate account of the hormonal regulation of gene expression.

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Recognition of myelin-associated glycoprotein by the monoclonal antibody HNK-1

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Myelin-associated glycoprotein (MAG) is a quantitatively minor component in both peripheral and central myelin sheaths that is thought to have a role in cell-cell interactions within the nervous system¹. We show here that a mouse monoclonal antibody, HNK-1, which is directed against human natural killer cells also recognizes an antigenic determinant of human central and peripheral nervous system white matter by immunoperoxidase staining of tissue sections. Immunoblot analysis of myelin proteins and purified extracted MAG indicates that the antigen recognized is MAG.

HNK-1 (Leu 7) is a monoclonal mouse IgM antibody which was produced against a membrane antigen from the cultured T-cell line, HSB-2. HNK-1 recognizes a differentiation antigen present on approximately 15% of normal peripheral blood lymphocytes^{2,3}. These cells are principally large granular lymphocytes and have been shown to contain most of the natural killer cell activity of the peripheral blood².

Sections of human nervous tissue were cut using standard histological technique. Immunoperoxidase staining⁴ was performed on these to detect recognition of the tissues by HNK-1. Control sections included those incubated with normal mouse serum (1:10) in place of specific antibody or the use of an irrelevant monoclonal mouse IgM at equivalent protein concentrations to HNK-1. Myelinated tissue from both central and peripheral nervous systems were strongly stained by HNK-1 (Fig. 1). Myelinated tissues from cerebral white matter, optic nerve, spinal cord and peroneal nerve all were specifically stained with HNK-1 (data not shown). Sections of kidney, adrenal, lymph node, liver and thymus were all negative when stained with HNK-1.

Crude myelin was prepared from fresh, normal human brain essentially by the technique of Norton and Poduslo⁵, and delipidated by extraction with either ether or ether:ethanol (3:2). The ether-insoluble fraction was termed myelin protein⁶. Myelin proteins were separated by SDS-polyacrylamide gel electrophoresis⁷, on 10 or 12% gels followed by either Coomassie blue staining or blotting onto nitrocellulose essentially by the technique of Towbin *et al.*⁸. On reacting the blots with HNK-1, a major band was observed with a mean molecular weight (MW) of 108,000 ± 3,500 (three determinations) (Fig. 2a, lane 3). A

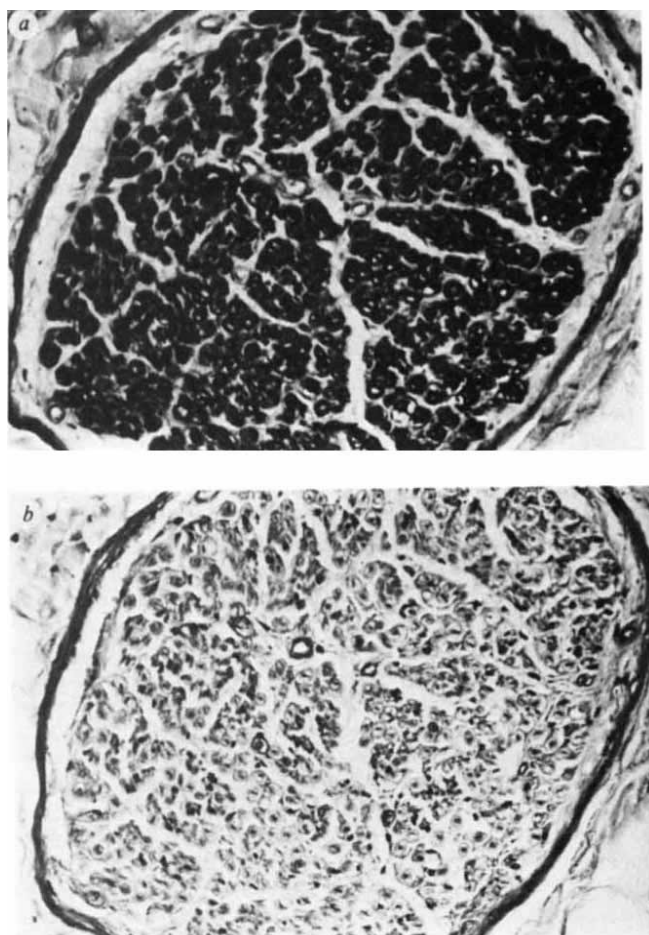


Fig. 1 Immunostaining of sciatic nerve by HNK-1. *a*, HNK-1, $\times 100$; *b*, control, $\times 100$.

Methods: Paraffin sections were prepared from formalin-fixed tissue using standard techniques. All sections were pretreated with either a 1:10 dilution of normal mouse serum or 3% bovine serum albumin before staining. Sections were incubated with each antibody preparation for 45 min. Control sections were treated with an irrelevant monoclonal mouse IgM at the same protein concentration as HNK-1. HNK-1 was used at a dilution of $5.0 \mu\text{g ml}^{-1}$ followed by rabbit anti-mouse IgM (1:50), swine anti-rabbit (1:50), rabbit peroxidase anti-peroxidase complexes (Cedarlane, 1:50 dilution) and finally reacted with diaminobenzidine⁴ for 4–6 min. The sections were then washed and counterstained with haematoxylin for 10 min and mounted.

series of minor bands, possibly representing antigen degradation components were also seen.

When myelin was extracted by the lithium diiodosalicylate-phenol procedure, a technique which selectively separates MAG from most other myelin proteins⁹, the component stained by HNK-1 was present in the extract (Fig. 2*a*, lane 4). The molecular weight of the antigen and its presence in the LIS-phenol extract suggested that the antigen being recognized was MAG. In an additional experiment, MAG was further purified by gel filtration on Sepharose CL-6B as recently described¹⁰. The purified MAG gave a single band after electrophoresis and staining with Coomassie blue (Fig. 2*b*, lane 1). When transferred to nitrocellulose sheets (Fig. 2*b*, lanes 2 to 4), the purified MAG also gave a corresponding single band stained with amido black (lane 2) or immune stained with HNK-1 (lane 3) or a known anti-MAG monoclonal antibody (lane 4). The latter IgG monoclonal antibody was obtained by the fusion of spleen cells from mice which had been immunized with isolated rat MAG¹¹. It is noteworthy that there were only 20 ng of MAG on lane 3 stained by HNK-1 (25-fold less than on lane 4), showing the high sensitivity of immune staining for MAG with HNK-1.

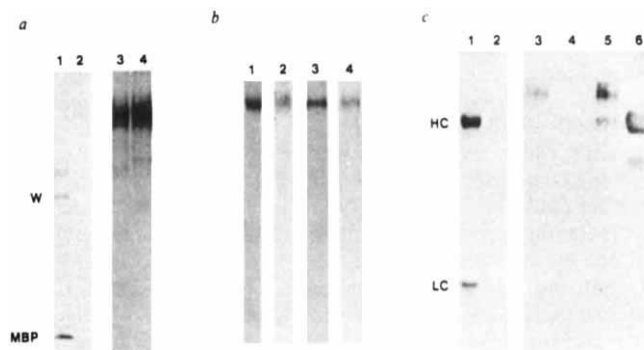


Fig. 2 Immune staining of myelin proteins on electroblots. After electrophoresis on SDS-polyacrylamide slab gels⁷, the proteins were transferred to sheets of nitrocellulose ($0.45 \mu\text{m}$ pore size, Millipore) essentially by the procedure of Towbin *et al.*⁸. Myelin was purified from human white matter by the procedure of Norton and Poduslo⁵. *a*, Protein staining and immune staining by HNK-1 of total myelin proteins and a lithium diiodosalicylate (LIS)-phenol extract of myelin. Human myelin was partially delipidated with ether:ethanol (3:2, v:v) to give the total myelin protein fraction. A partially purified preparation of MAG was obtained by extraction of myelin with LIS and partitioning against phenol as previously described⁹. Lane 1, 50 μg myelin protein; lane 2, 10 μg MAG; lane 3, 10 μg myelin protein; lane 4, 50 ng MAG. Lanes 1 and 2 were stained with amido black. Lanes 3 and 4 were reacted with HNK-1 (1:10,000 of 26 mg ml^{-1} stock), followed by peroxidase conjugated goat anti-mouse IgG-IgM (1:100, Boehringer-Mannheim), and the colour developed with 0.5 mg ml^{-1} diaminobenzidine and 0.01% hydrogen peroxide. MBP-myelin basic protein; w, major Wolfram protein. (Proteolipid is not seen because it does not transfer to the nitrocellulose with the blotting conditions used.) *b*, The MAG in a LIS-phenol extract was further purified by gel filtration on Sepharose CL-6B as recently described by Quarles *et al.*¹⁰ to give a single band on gel electrophoresis. The purified MAG was electrophoresed on each of the four lanes. Lane 1 is from an unblotted gel stained with Coomassie blue and contained 20 μg of the purified MAG. Lane 2 contained 20 μg of MAG, transferred to a nitrocellulose sheet and stained with amido black. Lane 3 contained 20 μg of MAG transferred to nitrocellulose and immune stained with HNK-1 as described above. Lane 4 had 500 ng of MAG transferred to nitrocellulose and stained as in lane 3 except the first antibody was a mouse monoclonal IgG prepared to MAG¹¹. *c*, Immunoprecipitation by HNK-1. Delipidated human myelin protein was solubilized with 1% (w/v) SDS at 6 mg ml^{-1} . A small aliquot containing 100 μg of protein was mixed with 100 μg of HNK-1 in phosphate-buffered saline/0.05% Tween-20 overnight at 4°C . The tubes were then spun at $20,000g$ for 30 min. The residue in the tube was resuspended in balanced saline¹⁸ and washed three times. The immunoprecipitate was then dissolved by boiling in 100 μl of Laemmli sample buffer and an aliquot was loaded on the gel. Two blots were prepared from the same gel by transferring for 2 h at 250 mA, removing the nitrocellulose sheet, placing a second sheet on the gel, and transferring again for 2 h. Lanes 1 and 2 were from an unblotted portion of the gel stained with Coomassie blue. Lanes 3 and 4 are from a blot and were reacted with rabbit anti-MAG (1:500)⁸ followed by peroxidase-labelled goat anti-rabbit IgG (1:1,000, Cedarlane). Lanes 5 and 6 are a blot of the same gel reacted with HNK-1 (1:500, 26 mg ml^{-1} stock) followed by peroxidase goat anti-mouse IgM (1:1,000, Cedarlane). Colour development on these blots was with 4-chloro-1-naphthol (Sigma). Lanes 1, 4 and 6, 10 μg of monoclonal mouse IgM control; lane 2, 10 μl aliquot of the immunoprecipitate; lanes 3 and 5, 2 μl aliquots of the immunoprecipitate. HC, heavy chain; LC, light chain.

With human myelin, it was possible to immunoprecipitate the myelin-associated protein antigen with HNK-1. Optimal immunoprecipitation was produced by combining approximately equal quantities of 1% SDS-solubilized myelin protein and HNK-1 antibody. Dilution of the SDS resulted in a final concentration of approximately 0.1%. After overnight incubation in the cold, the immune complexes were washed, solubilized

in Laemmli sample buffer and run on gels. Coomassie staining of the immunoprecipitates on gels confirmed the specificity of the immunoprecipitation reaction with none of the other myelin proteins appearing (Fig. 2c, lane 5). An extra band appeared at 43,000–45,000 MW, but this was probably a molecule such as actin which was nonspecifically brought down with the immune complexes and did not stain on the Western blots. This artefact could be minimized by inclusion of 0.05% Tween 20 in the immunoprecipitation mixture. The MAG band could not be seen on the Coomassie stained gels due to the low quantities present, but following blotting and staining with HNK-1, the bands visualized were found to comigrate with MAG (Fig. 2C, lane 5). Furthermore, on sequential blots of the same gel, the immunoprecipitated antigen could be stained with conventional rabbit anti-MAG¹² further indicating that MAG is the myelin antigen recognized by HNK-1 (Fig. 2C, lane 3). Control precipitation tubes containing either HNK-1 or myelin alone showed no bands on the gel indicating that the immunoprecipitation was specific (data not shown).

Myelin-associated glycoprotein (MAG) is a quantitatively minor myelin component characterized by Quarles *et al.*¹. Human MAG has a molecular weight of around 110,000, although incubation of myelin at pH 8.0 resulted in a rapid loss of MAG and the appearance of a band on SDS gels with an apparent molecular weight of 10,000 less than that of MAG¹³. The co-staining of partially purified MAG with HNK-1 and known anti-MAG antibodies on immunoblots is strong evidence for the identity of MAG and the HNK-1 antigen in white matter, but further work utilizing two-dimensional gels, and peptide maps is required. Evidence to support the close relationship of the antigen shared between the NK cell and myelin comes from the specific immunoprecipitation by HNK-1 of a 110,000 MW protein molecule from lysates of human peripheral blood lymphocytes¹⁴ although no detailed information concerning this antigen is yet available. Oger *et al.*¹⁵ have similarly proposed that suppressor T cells and ovine oligodendrocytes share an antigenic determinant. This work, however, has not been confirmed in other laboratories^{16,17}.

In conclusion, the antigen recognized by HNK-1 has been shown to be identical to or highly cross-reactive with MAG. The stability of MAG with regard to fixation and biochemical isolation, along with the ready availability of the monoclonal antibody, make this an ideal system for further biochemical, immunological, neurohistological and clinical investigation.

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An interleukin 1 like factor stimulates bone resorption *in vitro*

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Many activities are now ascribed to the monokine interleukin 1¹ including enhancement of immune responses, stimulation of thymocyte proliferation², activation of B cells³, stimulation of proteinase and prostaglandin production by connective tissue cells⁴, stimulation of the production of acute phase proteins⁵, induction of fever⁶ and the induction of neutrophilia⁷. These activities were thought to be due to various different factors, but are now considered probably due to very similar, if not identical, molecules^{8–11}. The term interleukin 1 (IL-1) was coined to describe the factor released by monocyte/macrophages which acts on T and B lymphocytes. Only after this definition had been accepted was it shown that target cells other than lymphocytes were affected by IL-1. Products of human blood monocytes (mononuclear cell factor, MCF) have been implicated in the pathogenesis of inflammatory diseases such as rheumatoid arthritis and periodontal disease^{12,13}. Bone resorption is often a feature of such diseases, and monocytes are frequently found at sites of localized bone resorption. Preliminary experiments with monocyte-conditioned medium indicated that MCF could stimulate bone resorption¹⁴. We therefore undertook this study to verify these observations and to determine whether purified IL-1 could stimulate connective tissue breakdown *in vitro*.

The IL-1 used in this study was prepared from normal monocytes and from leukocyte-rich samples from a single patient with chronic myelomonocytic leukaemia, under the care of Dr Jean Hester, Anderson Hospital, Houston, Texas. The adherent blood mononuclear cells from normal donors were cultured at $5\text{--}10 \times 10^5$ per ml in RPMI-1640 supplemented with 1% fetal calf serum and $0.1 \mu\text{g ml}^{-1}$ *Escherichia coli* 0111:B4 endotoxin for 40–48 h¹⁵. The supernatant was collected, concentrated on an Amicon YM10 membrane, and chromatographed on Sephadex G-75¹⁶. The unfractionated leukaemic cells (20% monocyte, 60% leukaemic, 9% polymorphonuclear (PNN) leukocyte, 10% lymphocyte) were washed in isotonic saline and cultured as above for 24 h at 2×10^7 per ml. The supernatant was fractionated as above, and the 12,000–20,000 molecular weight pool was further fractionated by isoelectric focusing in a sucrose gradient¹⁶. The presence of IL-1 was assessed by measuring the incorporation of ³H-thymidine by murine thymocytes cultured for 3 days in the presence of $1 \mu\text{l ml}^{-1}$ phytohaemagglutinin-P (the lymphocyte-activating factor (LAF) assay)⁸. Neither preparation contained detectable amounts of IL-2 as assessed by their failure to support the growth of IL-2-dependent T-cell lines. Bone resorption was assessed by measuring the release of ⁴⁵Ca from prelabelled mouse calvariae over a 48-h incubation period and expressing the release of ⁴⁵Ca as a percentage of the total bone isotope and then as a ratio of treated/control bones, as previously described¹⁷.

To establish the relationship between the two activities, fractions from both gel filtration and isoelectric focusing analyses were tested. After gel filtration, the only fractions which significantly stimulated calcium release were those eluting with an apparent molecular weight of 12,000–18,000 (Fig. 1). When the fractions were assayed at the same dilution for LAF, the two activities were found to elute coincidentally. The LAF and bone resorption activities also co-eluted when fractionated by

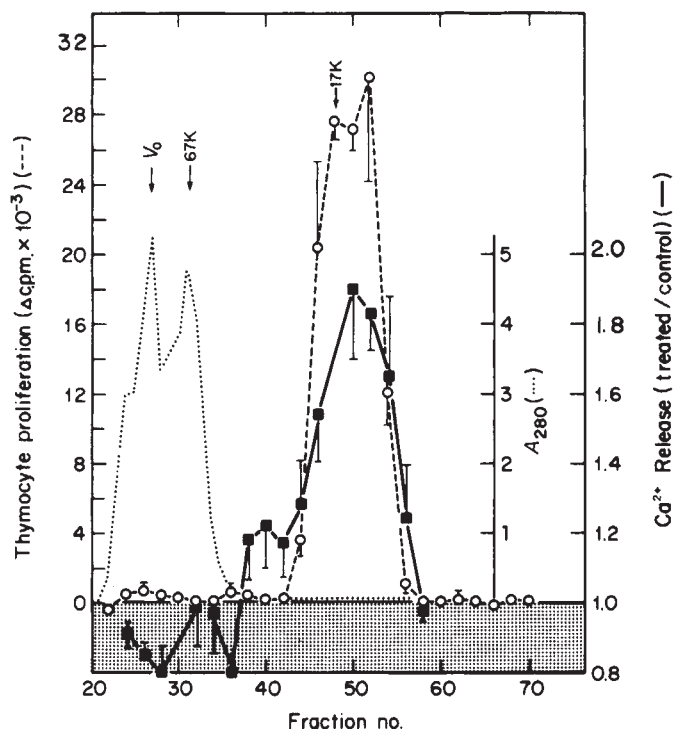


Fig. 1 Fractionation of LAF and bone resorption activities by Sephadex G-75 chromatography. The culture fluid concentrate of normal monocytes was chromatographed over Sephadex G-75 40–120 μm in 27 mM NaCl–1.6 mM phosphate pH 6.0 and the A_{280} monitored (dotted line). The elution position of the molecular weight standards (blue dextran, bovine albumin and myoglobin) are shown. Fractions were tested at a dilution of 0.25%. The bone resorption activity was assessed by culturing paired half-calvariae, prelabelled *in vivo* with ^{45}Ca , on stainless steel grids in 1.5 ml modified Bigger's medium (P_4)²⁹ for a 24-h preincubation period. Grids were then transferred to test media for 48 h, one half-calvaria acting as a control, the other as a test bone. The release of ^{45}Ca was measured and resorption expressed as a ratio of % released by treated/control bones (■). Fractions were tested for LAF activity as previously described⁸ (○). Thus C3H/HeJ thymocytes (1.4×10^6 per well) were cultured for 72 h in 0.2 ml RPMI-1640 supplemented with antibiotics, $5 \times 10^{-5}\text{M}$ 2-mercaptoethanol and 5% fetal calf serum in microwell trays with 1.25 μl phytohaemagglutinin P. Cultures were pulsed for the last 4 h with 0.5 μCi of 55 Ci mmol^{-1} ^3H -thymidine. The bars show 1 s.d.

isoelectric focusing (Fig. 2). The LAF activity at pH 6–6.7 is contributed by the molecule defined as IL-1¹⁸. The more acidic LAF is, however, frequently reported and when present exhibits all of the IL-1 activities for which it has been tested including LAF activity, BAF activity, induction of acute phase proteins, induction of fever and stimulation of prostaglandin release from synoviocytes and chondrocytes (unpublished results and refs 10, 11). Therefore, the coincidence of the bone-resorbing activity with both of the LAF active peaks argues strongly for the expression of both activities by the same molecule(s). A dose-response comparison of the two activities exhibited by the pI 6.3 fraction showed that a 50% increase in the rate of calcium release was induced by 4–20 LAF units, whereas 10 times more IL-1 caused an 80% increase in calcium release. (LAF at 5 units ml^{-1} stimulates half maximal thymocyte proliferation.)

The ability of IL-1 to stimulate acute phase protein release¹⁰, stimulate thymocytes¹⁹ and induce fever²⁰ is strongly inhibited by preincubation with phenylglyoxal, which reacts with the guanidinium groups of arginine residues. Table 1 shows that phenylglyoxal treatment of this preparation inhibited its activity in both the LAF and bone resorption assays. Based on the dose-response curve, 1% of the LAF activity and 2.5% of the bone resorption activity remained after treatment.

The results shown in Table 2 demonstrate that the stimulation of bone resorption by IL-1 was independent of prostaglandin

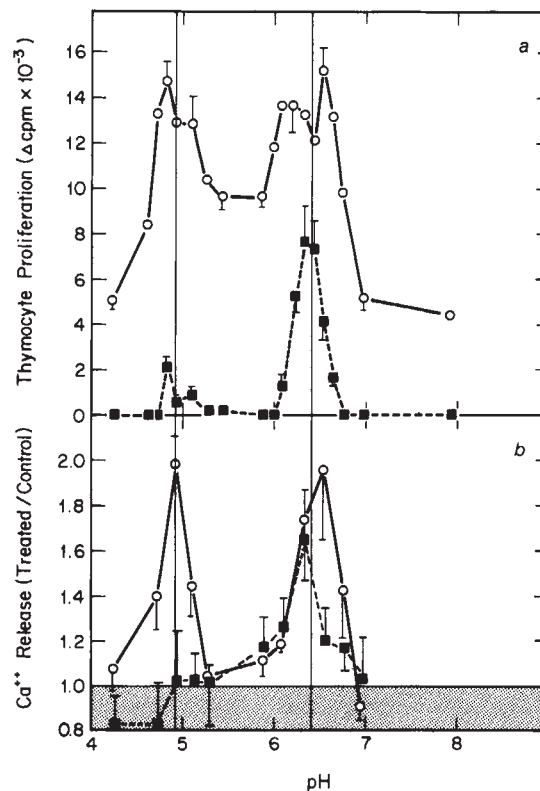


Fig. 2 Distribution of LAF and bone resorption activities on isoelectric focusing. The 12,000–20,000 molecular weight IL-1-rich fractions of a supernatant prepared from lipopolysaccharide-stimulated chronic myelomonocytic leukaemia leukocytes was fractionated by isoelectric focusing. *a*, LAF activity of the fractions measured by the thymocyte proliferation assay at dilutions of 0.25% (○) or 0.005% (■). *b*, Bone-resorbing activity measured at 0.2% (○) and 0.04% (■). No enhancement of bone resorption was observed at 0.005%.

Table 1 Treatment of IL-1 with phenylglyoxal

Additions to medium	% Release ^{45}Ca	Incorporation of ^3H -thymidine (c.p.m.)
None	13.8 ± 1.0	400
IL-1	30.8 ± 1.0	21,000
Phenylglyoxal-treated IL-1	16.6 ± 0.9	2,800

200 μl of IL-1 of molecular weight 15,000, pI 7.1 purified by gel filtration and isoelectric focusing was dialysed overnight against 200 mM imidazole pH 8.0. The sample was split into equal aliquots: an equal volume of 2% phenylglyoxal in 200 mM imidazole was added to one and buffer only to the other. Both were incubated at 25 °C for 4 h and passed over a 5-ml column of BioRad P4 equilibrated in RPMI-1640–5% fetal calf serum, collecting 0.5 ml fractions at 4 °C. The two peak fractions were pooled and tested for bone resorption and LAF activity at a 5% dilution.

synthesis by the bone cells. Incubation of the bones with IL-1 (100 units ml^{-1}) in the presence and absence of indomethacin (14 and 1.4 μM) produced no differences in the magnitude of the IL-1-induced stimulation of resorption.

The properties described for this IL-1-like material differ significantly from those of most of the factors which are known to stimulate bone resorption; however, its relation to osteoclast-activating factor (OAF) bears some consideration. OAF from various sources including human leukocytes²¹, myeloma cells²², tonsil lymphocytes²³ and certain lymphoid cell lines²⁴, has been partially characterized, and the data so far available suggest that this term probably describes a number of distinct although similar activities. The factor described here differs from these molecules in a number of ways. First, it is a monocyte rather

Table 2 Effect of indomethacin on IL-1-induced bone resorption

IL-1 (U ml ⁻¹)	Additive Indomethacin (µg ml ⁻¹)	⁴⁵ Ca release (%)	Treated/ control (%)
None	None	9.3 ± 0.64	
100	None	14.8 ± 1.59	1.59
None	0.5	6.6 ± 0.54	
100	0.5	11.0 ± 0.87	1.67
None	5.0	6.8 ± 0.54	
100	5.0	12.1 ± 0.61	1.78

IL-1 has a 15,000 molecular weight, pI 6.3 fraction derived from leukaemic leukocytes. All the responses differed from the control ($P < 0.05$), but there was no significant difference between the responses of the cultures receiving IL-1.

than a lymphocyte product³. Second, production of OAF from human leukocytes requires prostaglandin production by associated monocytes²⁵ whereas production of IL-1 is independent of prostaglandin synthesis¹². Third, the action of some OAFs is dependent on prostaglandin production by the stimulated bone cells²⁶, in contrast to our findings with IL-1.

Interleukin-1 therefore appears to be a new and distinct bone-resorbing agent which is as well, if not better, characterized than most species of OAF.

Although monocyte supernatants had previously been shown to stimulate the production of proteinases and prostaglandins by chondrocytes²⁷, synovial cells⁸ and bone cells²⁷, this is the first demonstration that IL-1 itself can alter the breakdown of bone. MCF and IL-1 can also stimulate the synthesis of extracellular matrix constituents by connective tissue cells. Our own preliminary results show that these agents can augment the synthesis of type I collagen and glycosaminoglycans by human osteoblast-like cells (J. N. Beresford, M. G. Gallagher and J. A. Gallagher, unpublished results) and the synthesis of glycosaminoglycans by synoviocytes (D. Hamerman, D.W. and E.J.I., unpublished results). IL-1 may therefore have a regulatory role in connective tissue metabolism. It may modulate both the resorptive osteoclasts and the synthetic activities of osteoblasts in such a way that it could be one of the mechanisms responsible for the coupling of the processes of bone destruction and bone formation which occur both in health and disease. In this regard, it is interesting that IL-1 has been found both in the joint fluids of patients with rheumatoid arthritis, a disorder characterized by excessive bone resorption, and of patients with osteoarthritis, who often have increased periarticular bone formation²⁸.

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Inversion in the *H-2* complex of *t*-haplotypes in mice

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Mouse *t*-haplotypes demonstrate strong linkage disequilibrium between *t*-lethal genes and specific *H-2* types, presumably a result of recombination suppression between *t* and normal chromosomes¹. The observation of free recombination occurring between two complementary *t*-haplotypes suggested a chromosomal mismatch between *t* and normal chromosomes². Recent data showing the *H-2* complex to be misplaced relative to two other markers, *T* and *tf*, in *t*-haplotypes³ suggested that chromosomal rearrangement in *t*-haplotypes might be the basis for their 'mismatch' with the normal chromosome. Here, to analyse the molecular nature of the rearrangement, we have cloned a polymorphic *H-2* class I restriction fragment, which had previously been shown to map centromeric to the serologically defined *H-2* complex in *t*-haplotypes⁴. Genetic mapping studies show that this cloned *t*-DNA is homologous to the *H-2 D* region of wild-type chromosomes, and that the *E_α Ia* gene maps telomeric to this DNA fragment in *t*-haplotypes, in contrast to its orientation in wild-type chromosomes. These results give molecular evidence for an inversion of *H-2* in *t*-haplotypes, which may be at least partially responsible for recombination suppression and thus for linkage disequilibrium.

Previously, we identified restriction fragments containing *H-2* class I sequences which were polymorphic between the *t^{w32}* and *t^{w12}* (= *Ti^{s6}*) haplotypes. Using a panel of recombinant chromosomes derived from those two parental *t*-haplotypes, we found that the polymorphic fragments were allelic to each other and mapped centromeric to the serologically defined *H-2* complex in *t*-haplotypes⁴. This finding was interesting because in wild-type chromosomes 17 no *H-2* sequences have been mapped to the left of the *H-2* complex; they map to *H-2* or telomeric to it in the *Qa-Tla* region⁵⁻⁷.

We cloned the polymorphic fragment from the *t^{w32}* haplotype in the λ phage L47.1, as described in Fig. 1 legend. To verify that the 21-kilobase (kb) fragment cloned corresponded to the polymorphic fragment in *t^{w32}*, clone DNA (φ7.1) was *EcoRI* restricted and electrophoresed in an agarose gel along with *EcoRI* digests of the *t^{w32}* and *t^{w12}* DNA. Hybridization of the transferred DNA⁸ with the *H-2* cDNA probe⁹ confirmed that the cloned DNA co-migrated with the 21-kb polymorphism fragment in *t^{w32}* as shown in Fig. 1a. This point was further verified by using a subfragment of the clone known to be free of highly repetitive sequences (1.6-kb *EcoRI*-*HindIII*; see Fig. 1c) as a probe (Fig. 1b).

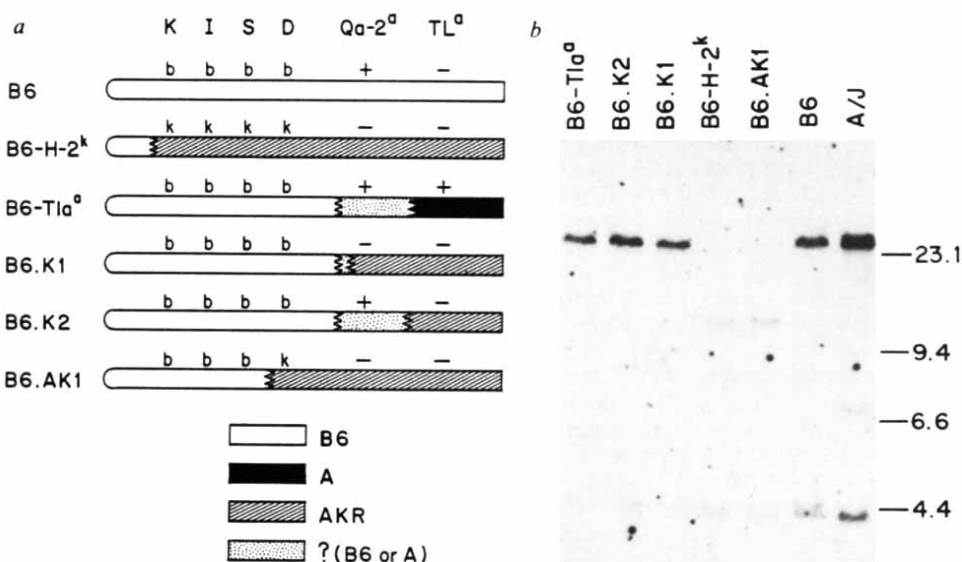
Figure 1c shows a partial restriction map of φ7.1. An *H-2* class I sequence is found in the middle of the clone as determined by hybridization with the cDNA probes pH-2 III and pH-2 IIa, allowing the clone to be oriented. To investigate the possibility that DNA from *t*-haplotypes may contain structural peculiarities, we examined the nature and distribution of repetitive sequences in the cloned DNA using high-complexity

Fig. 1 Characterization of clone $\phi 7.1$: a 21-kb *EcoRI* fragment isolated from t^{w32} haplotype. *a*, Verification of the identity of the cloned DNA and the 21-kb *EcoRI* fragment in t^{w32} DNA. *EcoRI* digests of $\phi 7.1$ and DNAs of different strains were hybridized with *H-2* cDNA probe pH-2 III. Tt^{s6} and t^{w32} are heterozygotes with C3H for the *t* region of chromosome 17. (Homozygotes of Tt^{s6} or t^{w32} are lethal.) Tt^{s6}/t^{w32} is a heterozygote of Tt^{s6} and t^{w32} and does not have C3H components. The t^{w12} type band (23 kb) and the t^{w32} type band (21 kb) are labelled. *b*, Hybridization of the 1.6-kb *EcoRI*-*HindIII* fragment from $\phi 7.1$ (see *c*) to the same set of DNAs as in *a*. The filter was washed more stringently, that is, 68 °C, 0.1 $\times$ SSC/0.1% SDS, 20 min. *c*, Organization of clone $\phi 7.1$. Hatched boxes (▨) are fragments containing *H-2* sequences that hybridized with pH-2 III or pH-2 IIa, respectively. Open boxes (□) are vector arms. Regions which do not contain highly repetitive sequences are indicated by 'non-repeat'.

The 1.6-kb *EcoRI*-*HindIII* fragment was used as a unique copy probe. Restriction sites are shown as strokes for the four enzymes. The exact positions for two additional *HindIII* sites were not determined. *d*, Comparison of C3H and C3H. t^{w32} for repetitive sequences in $\phi 7.1$. $\phi 7.1$ was digested with the enzymes shown, electrophoresed and blotted on nitrocellulose paper. Two filters were prepared in identical ways. Each filter was hybridized with 32 P-labelled C3H or C3H. t^{w32} genome DNA in conditions where only repetitive DNA sequences would be detected. H, digested with *HindIII*; B+H, digested with *HindIII* and *BamHI*; B, digested with *BamHI*. The left panel was hybridized with C3H DNA and the right panel with C3H. t^{w32} DNA.

Methods: Approximately 1 mg of high molecular weight liver DNA was digested to completion with *EcoRI* and then size fractionated in a circular 0.75% agarose gel in a preparative electrophoresis apparatus (Bulls Eye Gel Apparatus, Hoefer Scientific, San Francisco). Fractions were electrophoresed, transferred to a nitrocellulose filter and then hybridized with *H-2* cDNA probes. DNA of the fractions enriched for the 21-kb *H-2*-containing fragments were cloned into phage L47.1 according to described procedures¹⁶. Phage plaques were transferred to nitrocellulose filters. Filters were base denatured, neutralized, baked at 80 °C for 3 h and then hybridized with pH-2 III⁹ which was nick-translated¹⁷ to a specific activity of about 2×10^8 c.p.m. per μ g by using [α - 32 P]dCTP at 3,000 Ci mmol⁻¹. Nick-translated probes were base denatured and used at 10^6 c.p.m. ml⁻¹ hybridization buffer. Hybridization was carried out at 40 °C overnight in a buffer consisting of 50% formamide, 5 $\times$ SSC, 7 mM Tris pH 7.5, 1 $\times$ Denhardt's solution, 25 μ g ml⁻¹ salmon sperm DNA, and 10% dextran sulphate. The same condition was used for hybridizing genome DNA blots and cloned DNA blots, and for screening recombinant phage plaques. Hybridized filters were washed twice in 2 $\times$ SSC/0.1% SDS at room temperature for 20 min, and then twice in 0.1 $\times$ SSC/0.1% SDS at 52 °C for 20 min. For a more stringent condition, filters were additionally washed once at 68 °C in 0.1 $\times$ SSC/0.1% SDS for 20 min. Blots were autoradiographed on Kodak XAR film with use of a DuPont Cronex intensifying screen.

Fig. 2 Mapping the unique-copy probe of clone $\phi 7.1$ to wild-type chromosomes. *a*, Chromosomal map of the *H-2* complex of B6 and B6 congenic mice (modified from ref. 18). The origin of chromosome for regions K, I, S and D is labelled either with 'b', from B6, or with 'k', from AKR mice. For *Qa-2*, 3 and *Tla* regions, the presence or absence of the *Qa-2*^a or *Tla*^a allele is designated (+) or (-). B6-*H-2*^k has the entire *H-2* complex donated from an AKR mouse. B6-*Tla*^a contains the *Tla* region from strain A. B6.K1 and B6.K2 arose from recombination between B6-*H-2*^k and B6-*Tla*^a. B6.AK1 is a recombinant between B6-*Tla*^a and B6-*H-2*^k at a site between the S and D regions. The origin of the dotted region is unknown because B6 and A strains carry *Qa-2*^a. B6, B6.K2 and B6.AK1 were from the mouse colonies at the Center for Laboratories and Research of the New York State Department of Health at Albany. Other mice were gifts from E. A. Boyse. *b*, Hybridization of *EcoRI* digests of mice described in *a* with 32 P-labelled 1.6-kb *EcoRI*-*HindIII* fragment of $\phi 7.1$. The filter was washed at high stringency; 68 °C, 0.1 $\times$ SSC/0.1% SDS, 20 min.



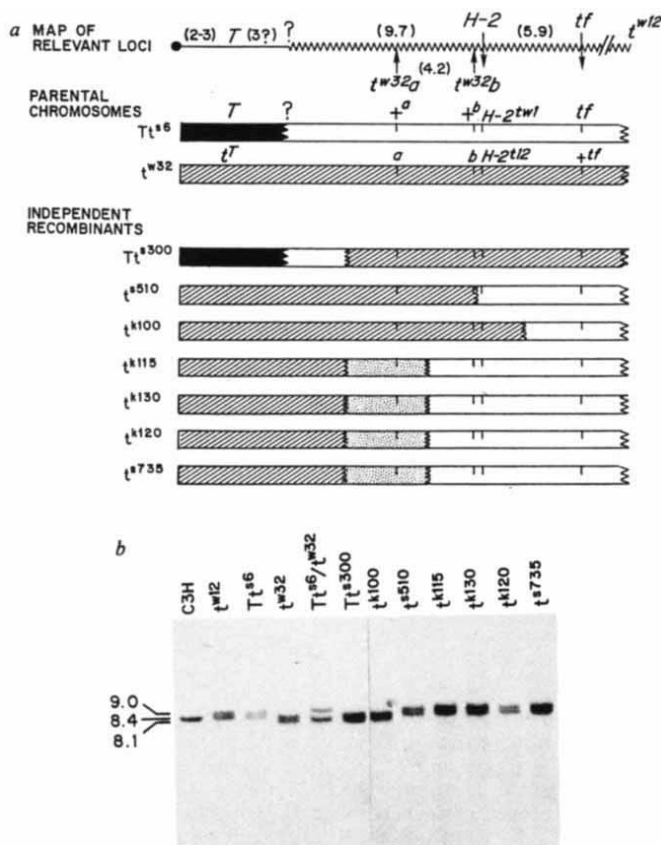


Fig. 3 Mapping the E_α gene to t -haplotypes. *a*, Chromosome constitution of parental and recombinant mice. Top, map positions of relevant loci. One of the parents, T^{16} , is a recombinant consisting of normal chromosome at the centromeric end followed by distal t^{w12} chromosome². The other parent, t^{w32} , is a complete t -haplotype. ■, Normal chromosome; □, t^{w12} chromosome; ▨, t^{w32} chromosome; ▩, undetermined, t^{w12} or t^{w32} . *b*, Hybridization of *Hind*III digests of DNA from parental and recombinant mice with ³²P-labelled E_α sequence. The E_α probe is a 3.4-kb *Sal*I fragment from the clone 32.1 subcloned in pBR325¹⁰. C3H DNA is included as a control. The t^{w12} type (9.0 kb), C3H type (8.4 kb) and t^{w32} type (8.1 kb) bands are labelled on the left of the gel. T^{16}/t^{w32} is a heterozygote of the two parents, and does not contain the C3H band.

genome DNA as probes. As shown in Fig. 1d, both probes, C3H and C3H. t^{w32} DNA, detected common DNA fragments, suggesting that repetitive sequences in $\phi 7.1$ are common to both t -haplotype and wild-type DNA.

Because $\phi 7.1$ was derived from a class I $H-2$ sequence that mapped to the left of the serologically determined $H-2$ complex in t -haplotypes, it was necessary to map this sequence in wild-type chromosomes. The 1.6-kb *Eco*RI-*Hind*III fragment from $\phi 7.1$, free of highly repetitive sequences, was used as a probe against DNAs of a panel of C57BL/6 congenic strains of mice. The chromosomal composition of each strain differs for a defined region of the $H-2$ complex (Fig. 2a), permitting the localization of any polymorphic fragments to a specific region of the $H-2$ complex.

Using stringent washing conditions, we reduced the complexity of cross-hybridizing species to a single major band with the 1.6-kb *Eco*RI-*Hind*III probe. As shown in Fig. 2b, a polymorphism was detected between B6 and B6- $H-2^k$ (24 kb for B6 and 13 kb for B6- $H-2^k$). B6.AK1 has the same fragment as B6- $H-2^k$, leading to the conclusion that the probe used is detecting $H-2$ sequences to the right of the S region of the $H-2$ complex. Comparison of the polymorphic fragments detected between B6, A/J and B6- Tla^a maps the sequence to the left of the TL region, leaving D or Qa as the only possible map positions for this sequence. Finally, a comparison of B6.K1 and

Table 1 Panel typing with anti- t^{s510} and monoclonal antibody anti- t^{w12}

	t^{k115} anti t^{s510}	Monoclonal anti t^{w12} (212i:4:2)
Haplotype		
T^{16}/t^{w32}	<25	2,500
t^{w32}	200	<50
T^{300}	200	<50
t^{s510}	200	2,500
t^{k100}	200	<50
t^{k115}	<25	2,500
t^{k735}	<25	2,500

'Anti- t^{s510} ' was made by immunizing (C3H/DiSn $\times$ T/ t^{k115}) normal-tailed F_1 with (C3H/DiSn $\times$ T/ t^{s510}) normal-tailed F_1 skin grafts initially and lymphoid cells at biweekly intervals thereafter. Sera from three good responders were pooled after hyperimmunization. This reagent kills 100% of lymph node and thymus cells, and is negative on a variety of TL^+ and Qa^+ strains (B6, A.CA, P/J, BALB/c and A/J). '212i:4:2' is a monoclonal antibody made by fusing the spleen of C3H/DiSn immunized with lymphoid cells of C3H. t^{w12} with myeloma SP-2 (Andrew Balber, manuscript in preparation). Sepharose Staph A purified supernatant is negative on C3H ($H-2^k$) and BTBRTF/Nev ($H-2^b$) lymph node cells at 1:50 and has a cytotoxic titre of 1:50,000 on $H-2^b$ or $H-2^k/t^{w12}$, t^{w71} , t^{w73} , t^{s6} and t^{lub1} ; 1:2,500 on $t^{w12}tf$. It is negative at 1:50 on $H-2^k$ or $H-2^b/t^{s510}$, t^{s1} , t^{s2} , t^{w32} , t^{w5} , t^{w2} , t^{wPA-1} . Complement mediated cytotoxicity was done as in Artzt *et al.*³. Numbers are the reciprocal of titre. All haplotypes were heterozygous with C3H/DiSn ($H-2^k$).

B6.K2 with B6 and B6- $H-2^k$ shows that the 1.6-kb *Eco*RI-*Hind*III probe derived from t -haplotype DNA maps to the $H-2D$ region in wild-type chromosomes.

The results described above suggested a rearrangement of the $H-2$ complex in t -haplotypes. To analyse this further, we used another unique-copy DNA sequence, the E_α *Ia* gene sequence, as a reference point¹⁰. The 3.4-kb *Sal*I fragment from a cosmid clone of BALB/c genome contains exons of the E_α gene. *Hind*III digests probed with E_α revealed a polymorphism between the t^{w32} and t^{w12} (or T^{16}) haplotypes. To map this polymorphism, a series of recombinants between t^{w32} and T^{16} (Fig. 3a) was used. As shown in Fig. 3b, when probed with the E_α sequence, t^{s510} yielded the t^{w12} -type *Hind*III fragment (9.0 kb) whereas t^{k100} contained the t^{w32} -type fragment (8.1 kb) in addition to the common C3H 8.4-kb fragment. Thus, the E_α gene maps to the region right of the cross-over point in the t^{s510} recombinant. When the 1.6-kb *Eco*RI-*Hind*III fragment was used as a probe against t^{s510} DNA, it mapped to the left of the cross-over point (data not shown), as would be expected from previous studies⁴. These results establish the gene order as: centromere- $H-2D$ region- E_α gene in t -haplotypes, as distinct from wild-type chromosomes in which the gene order is: centromere- E_α - $H-2D$.

The molecular mapping of the $H-2D$ region of t^{w32} to the left of E_α and to the left of the recombinational breakpoint in t^{s510} raised the question of why we had failed to detect serological specificities relevant to $H-2^{w32}$ in our original definition of t^{s510} , which we scored as $H-2^{w12}$ -positive, $H-2^{w32}$ -negative³. One possibility was that the antiserum defining the $H-2$ type of t^{w32} lacked specificities against the $H-2D$ region products of t^{w32} . The antiserum was generated in BALB/c ($H-2^d$) against $H-2^b/H-2^{w32}$, and absorbed with cells of $H-2^b$ and $H-2^b/H-2^{w12}$ mice; thus, specificities common to t^{w32} and $H-2^b$ or $H-2^{w12}$ could have been removed. Alternatively, the t^{w32} -derived $H-2D$ region left of the cross-over point in the recombinant t^{s510} might contain only pseudogenes.

These two possibilities were tested by immunizations designed to detect any $H-2$ antigen encoded in the chromosomal region to the left of the cross-over point in t^{s510} . We immunized t^{k115} recombinants with t^{s510} cells because t^{k115} differs from t^{s510} only for a limited region to the left of the $H-2$ complex, where $\phi 7.1$ maps (Fig. 3a). This immunization produced a typical

anti-H-2 antiserum directed against the t^{w32} haplotype (Table 1 legend). The chromosomal region coding for this H-2 antigen also mapped to the left of the cross-over point in the t^{s510} recombinant (Table 1), as would be expected. The same recombinants were typed with a monoclonal antibody (212i.4.2) made against the t^{w12} chromosome. This typing served as an additional confirmation of the presence of t^{w12} specificity to the right of the breakpoint on t^{s510} , since 212i.4.2 reacts only with t -haplotypes containing the t^{w12} H-2 type. These results indicate that the H-2D region of the t^{s510} haplotype mapping to the left of the E_α gene does actually code for H-2 products. Thus, the gene order is inverted in t^{s510} compared with that in wild type.

Two other intra-H-2 recombinants derived from another set of parental t -haplotypes have been analysed with probes for H-2D and E_α and show the same inverted gene order (Eric Lader, personal communication). Thus, the inversion of H-2 in t -haplotypes appears to be a general one.

Recently, we showed by serological analysis that the H-2 complex is in an anomalous position in t -haplotypes with respect to the genetic markers, T and tf , and is located amongst several non-allelic, but functionally related, t -lethal genes^{3,11}. Thus, the gene order is, from the centromere, T - tf -H-2 in wild type, but T -H-2- tf in t -haplotypes. One explanation was that t -haplotypes contain a simple chromosomal rearrangement such as an inversion. This explanation could also help to account for recombination suppression between t -haplotypes and normal chromosomes. However, neither a search for anaphase I bridges¹² nor electron microscopic analysis of synaptonemal complexes¹³ in $t/+$ males has given evidence for an inversion of substantial length. It has been suggested that the anomalous map position of H-2 might be due to cryptic gene expression, so that generally unexpressed class I H-2 sequences are expressed in t -haplotypes, whereas the sequences normally expressed in wild-type chromosome 17 are silent¹³. Such a model is unlikely as no class I H-2 sequences have been mapped to the left of the tf locus in wild-type chromosome, whereas the majority were shown to map to H-2 itself or to the right of H-2, that is, to Qa and TL (refs 5-7). The results described here show the existence of a physical rearrangement in t -haplotypes which is most simply explained by an inversion, the extent of which is unknown, although other mechanisms, such as gene conversion, could generate similar results.

Observations suggesting rearrangement in t -haplotypes has been recently presented¹⁴. In this case it was found that whereas two inbred strains contained multiple copies of Qa -2,3 region homology units, four t -haplotypes contained only a single copy. However, since the regions flanking the single copy of t -haplotypes had restriction maps unrelated to those in wild type, no information on the nature of the rearrangement was obtained.

Differences in moderately repeated sequences have been proposed as being responsible for recombination suppression between t and wild-type chromosomes¹⁵. However, at least in the experimental conditions we used, we did not find any repetitive sequences unique to t -DNA in our clone. Our results, however, do not rule out the possibility that other unique repetitive sequences exist elsewhere in t -DNA.

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A new H-2-linked class I gene whose expression depends on a maternally inherited factor

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The maternally transmitted antigen (Mta) is expressed on the cells of most strains of mice^{1,2}. It is a medial histocompatibility antigen³, that is, it is recognized by unrestricted cytotoxic T lymphocytes as a major H antigen, but unlike these it is a weak transplantation antigen and does not itself restrict the T-cell recognition of minor H antigens. All other medial H antigens are encoded by genes closely linked to the major histocompatibility complex³, H-2 in the mouse. By contrast, Mta appeared to follow extrachromosomal, maternal inheritance. Several substrains of NZB, NZO and non-inbred European NMRI mice are Mta-negative. Females of these strains bear only Mta⁻ offspring, while females of the inbred Mta⁺ strains bear only Mta⁺ offspring. Repeated backcrossing from Mta⁺ females to NZB or NMRI males has shown that, given the right cytoplasmic genes, the chromosomal genes of these Mta⁺ strains permit expression of Mta⁺. As the Mta type of a mouse cannot be influenced by embryo transfer or foster nursing^{2,4}, we concluded that it was determined by a cytoplasmic factor (*Mtf*), transmitted through the egg. We now show that a gene, *Hmt*, closely linked to the H-2 complex, is also required for expression of Mta.

Because segregation of Mta types within litters had never been observed, we were puzzled when among three mice from a stock of *Mus musculus castaneus*, two were Mta⁺ and one Mta⁻ (ref. 2). Another seven mice, all from different mothers (from the non-inbred stock of *M. m. castaneus* of the Roswell Park Memorial Institute) were all Mta⁻. These Mta⁻ mice had mitochondrial DNA of the type found in Mta⁺ laboratory mice (ref. 5 and M. Hiram, personal communication), suggesting that their maternally defined cytoplasm might have been permissive for expression of Mta but that they carried a negative allele of a chromosomal gene. To test this hypothesis, we crossed such *M. m. castaneus* with previously characterized strains of mice and typed the hybrid progeny for Mta (Fig. 1).

The control groups in Table 1 behaved as expected: Mta⁺ C57BL/6 and C3H females bore only positive and NMRI/Bom females only negative offspring when mated to Mta⁻ *M. m. castaneus* males. On the other hand, all F₁ hybrids bred from the Mta⁻ *M. m. castaneus* females were Mta⁺, even when a cytoplasmic Mta⁻ strain (NMRI/Bom or NZO/Dus) was used as the male parent. We thus observe that two Mta⁻ strains can complement each other and yield positive offspring.

To test whether the *castaneus* mice had a negative allele of a single chromosomal gene required for expression of Mta, (*M. m. castaneus* ♀ × C3H♂)F₁ females were backcrossed to Mta⁻ *M. m. castaneus* males. Mta segregated in a ratio indistinguishable from 1:1. This defines the chromosomal gene *Hmt* (for

Table 1 Mta typing of offspring from crosses with *M. m. castaneus*

♀	Mta	Parents ♂	Mta	Offspring Mta
NMRI/Bom	-	<i>M. m. castaneus</i>	-	16-/0+
C57BL/6	+	<i>M. m. castaneus</i>	-	0-/7+
C3H.Pgk-1 ^a	(+)*	<i>M. m. castaneus</i>	(-)*	0-/5+
<i>M. m. castaneus</i>	-	<i>M. m. castaneus</i>	-	8-/0+
<i>M. m. castaneus</i>	(-)*	C3H.Pgk-1 ^a	(+)*	0-/5+
<i>M. m. castaneus</i>	-	B10.WB(69NS)/Sn	+	0-/8+
<i>M. m. castaneus</i>	-	NZO/Dus	-	0-/8+
<i>M. m. castaneus</i>	-	NMRI/Bom	-	0-/3+
B10.CAS2	-	NMRI/Bom	-	0-/7+
B10.CAS2	-	NZB/Bom	-	0-/6+

* The cross was made at RPMI, and only the offspring were typed.

Table 2 Mta and H-2 segregation in F₂ progeny from matings of *M. m. castaneus* and C3H.Pgk-1^a

	H-2D (H-2K)*			Total
	k/k	k/c	c/c	
Mta ⁺	32 (32)	73 (72)	2 (3)	107
Mta ⁻	0 (0)	1 (4)	38 (35)	39
Total	32 (32)	74 (76)	40 (38)	146

F₂ mice were splenectomized, and their spleen cells were typed for Mta as described in Fig. 1 legend, always using at least two killer-target combinations which differed at H-2^{1,2,4}. The spleen cells were H-2 typed by measuring the binding of monoclonal antibodies 3-83 and 15-5-5, specific for H-2K^k and H-2D^k of C3H.Pgk-1^a (ref. 18), and 34-2-12 (ref. 19), which reacted with the H-2D of the *castaneus* mice (symbolized by c). H-2D segregation (1:2:1): $\chi^2 = 0.23$, $P = 0.633$; Mta segregation (3:1): $\chi^2 = 0.90$, $P = 0.636$. Independent segregation of H-2D and Mta: $\chi^2 = 146.05$, $P < 10^{-4}$.

Table 3 Genotype and Mta phenotype of representative mice

Strain	Genotype		Phenotype
	Cytoplasmic	Chromosomal	
C57BL/6, C3H.Pgk-1 ^a	<i>Mtf</i> ^a	<i>Hmt</i> ^{a/a}	Mta ⁺
B10.CAS2, <i>M. m. castaneus</i>	<i>Mtf</i> ^a	<i>Hmt</i> ^{b/b}	Mta ⁻
NZO/Dus, NMRI/Bom	<i>Mtf</i> ^b	<i>Hmt</i> ^{a/a}	Mta ⁻
(<i>M. m. cast.</i> × NMRI)F ₁	<i>Mtf</i> ^a	<i>Hmt</i> ^{a/b}	Mta ⁺
(NMRI × <i>M. m. cast.</i>)F ₁	<i>Mtf</i> ^b	<i>Hmt</i> ^{a/b}	Mta ⁻

histocompatibility, dependent on a maternally transmitted factor).

As Mta is similar to the H-2-linked Qa antigens in its immunological properties^{3,6}, we also tested the backcross mice for linkage between *Hmt* and H-2. Of 40 mice typed (23♀ and 17♂), 21 were positive for H-2^k and Mta, 19 were negative for both, and no recombinants were found. This sets an upper (95% confidence) limit to the recombination frequency in females of 7.2%. We have further examined 67 F₂ hybrids bred from two pairs of (*M. m. castaneus* × C3H)F₁ mice and 79 bred from two pairs of the reciprocal (C3H × *M. m. castaneus*)F₁. The H-2 haplotypes of the *castaneus* mice in the two crosses were not identical, but as no significant differences were found in the segregation ratios, the results have been pooled in Table 2. Mta follows the expected 3:1 ratio and shows close linkage to H-2.

Three recombinational events separated *Hmt* from both H-2K and H-2D. Another recombinant was negative for H-2K^k and positive for H-2D^k and Mta, while three others were positive for H-2K^k and negative for H-2D^k and Mta. Thus, all recombinants place *Hmt* in the *Tla* region, 2.0 ± 1.2 centimorgans from H-2D, 4.6 ± 1.8 centimorgans from H-2K. Several clusters of H-2 class-I-like genes are known to occupy this stretch of chromosome 17 (ref. 7). Six of the seven recombinants are alive and are being progeny tested. Typing for isozymes and serological markers should allow us to map *Hmt* more precisely relative to known loci.

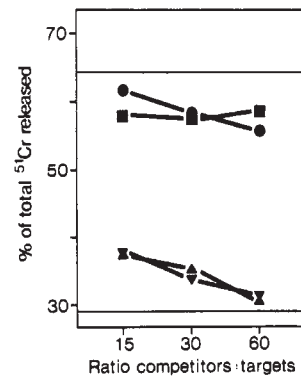


Fig. 1 Complementation between Mta⁻ parents to yield Mta⁺ offspring, shown by competitive inhibition of killing. Mta-specific killer cells (from an Mta⁻ H-2^b mouse, immunized *in vivo* and restimulated *in vitro* with Mta⁺ H-2^b cells) were mixed 30:1 with ⁵¹Cr-labelled target cells from a C3H/HeJ mouse (Mta⁺ H-2^k). The lower horizontal line indicates the release of ⁵¹Cr in the absence of killers and the upper line in the absence of competitors. Spleen cells from the parental *M. m. castaneus* ♀ (●) and NMRI/Bom ♂ (■) and from their F₁ progeny, one ♀ (▼) and one ♂ (▲), were stimulated with concanavalin A and the lymphoblasts were added to the killer-target mixture in the ratios indicated. The Mta⁺ F₁ cells compete effectively for the killers and prevent the lysis and subsequent release of ⁵¹Cr from the labelled target cells, whereas the Mta⁻ parents are ineffective.

The number of recombinants found between H-2K and H-2D was higher (4/186 or 2.15%, as against 29/8811 or 0.33%) than has been reported in the literature⁸. Markers on chromosomes 7 and X that have been followed in crosses of *M. m. castaneus* have not recombined at elevated frequencies^{9,10}. The frequency of recombination between H-2K and H-2D depends strongly on the haplotypes involved^{8,11}, and the same may well hold for the immediate neighbourhood of H-2.

A third H-2 haplotype associated with a negative allele of *Hmt* was found in the H-2 congenic Mta⁻ strain B10.CAS2. B10.CAS2 was produced by J. Klein (personal communication) by crossing a *M. m. castaneus* male with a B10.BR female and backcrossing for 10 generations to B10.BR with selection for the Ss^h allele. The chromosomal segment retained from the *castaneus* parent extends from the proximal side of H-2K to the distal side of *Tla*¹². Not all *M. m. castaneus* are Mta⁻, however. Several mice from the Roswell Park colony and the inbred strain CAST/Ei, all of which differed at H-2 from the Mta⁻ mice described here, were Mta⁺. All the *castaneus* lines were descended from a small number of mice trapped by J. T. Marshall in Thailand in the 1970s¹³.

In addition to *Hmt*, a functional *B2m* gene is required for detection of Mta. The R1 cell line from strain C58/J is Mta⁺, but its variant R1(TL⁻) that lacks β₂-microglobulin (β_{2m}) is thus Mta⁻ (ref. 3). Further evidence that β_{2m} is associated with Mta is shown in Fig. 2. A monoclonal antibody, anti-Ly-m11, specific for the β_{2m} of C57BL mice¹⁴⁻¹⁶, inhibits the Mta-specific killing of target cells with the *B2m*^b allele, but not of targets with the *B2m*^a allele. In our hands the same antibody had no significant effect on H-2-specific killing, perhaps because it is a more heterogeneous response. A mixture of monoclonal antibodies specific for H-2K and H-2D never interfered with lysis specific for Mta, but blocked H-2-specific killing. These results were confirmed in several experiments, on different target cells (H-2^k and H-2^b) and using different killer cells (long-term cultured lines or cells obtained fresh on day 5 of mixed lymphocyte culture). Differences in affinity or fine specificity may explain why another monoclonal anti-β_{2m}, raised in the same strain combination¹⁷, failed to inhibit Mta-specific killing.

Table 3 summarizes our findings. Beside the requirement for β_{2m}, two factors determine whether Mta is expressed. All mice with the maternally inherited factor *Mtf*^b are negative. Mice

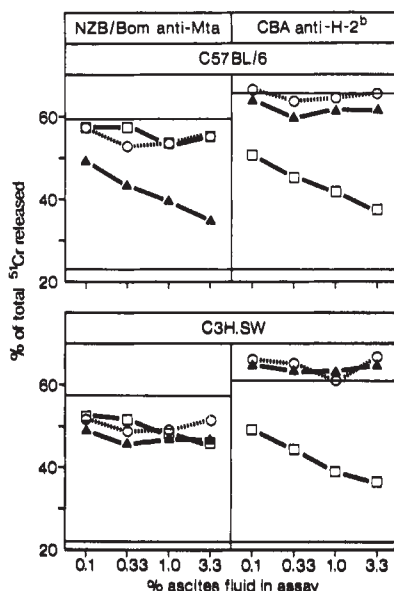


Fig. 2 Inhibition of Mta-specific killing by monoclonal antibodies. Normal mouse serum (○), anti-Ly-m11 monoclonal antibody specific for β_2 -microglobulin from B2m^b mice (▲)¹⁴, or a mixture of monoclonal antibodies B8-24-3 and H141-51 specific for H-2K^b and H-2D^b (□)^{20,21} was added to the ⁵¹Cr-labelled target cells before the killers and was present over the 3.5-h assay²². The concanavalin A-stimulated target cells were from C57BL/6 (Mta⁺ H-2^b B2m^b) or C3H.SW (Mta⁺ H-2^b B2m^a) mice. The lower horizontal line shows the release of ⁵¹Cr in the absence of killer cells, the upper line in the absence of added antibodies. NZB/Bom T killer cells (Mta⁺ H-2^d B2m^a) had been maintained for 6 weeks in culture by continued restimulation with congenic Mta⁺ cells in the presence of supernatant from concanavalin A-stimulated rat spleen cells. They were assayed at a ratio of three killer cells per target cell. Killers specific for H-2^b were from a 5-day mixed lymphocyte culture of CBA/J spleen cells (Mta⁺ H-2^k B2m^a) stimulated with C3H.SW, and were assayed at a ratio of 30 initial responder cells per target cell.

with *Mtf*^a are positive only if they have at least one positive allele (a) of the H-2-linked gene *Hmt*. It seems likely that *Hmt* is the structural gene for Mta, and that Mta belongs to class I of histocompatibility antigens, that is, a 40–50,000 molecular weight heavy chain associated with β_2 m.

The role of the cytoplasmically inherited factor is unknown. *Mtf*^b strains have a unique type of mitochondrial DNA⁵, and thus a mitochondrial gene could be involved. The cytoplasmic factor might determine whether the product of the *Hmt* gene is expressed at all, or expressed in an antigenically modified form. Alternatively, *Mtf* might be a cytoplasmically inherited defective virus, coding for an antigen which is recognized in association with the product of *Hmt*.

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Amplification of a gene coding for human T-cell differentiation antigen

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Using previously isolated mouse L-cell transferents for the human T-cell differentiation antigen Leu-2, we now report the first example of spontaneous gene amplification for membrane antigens. The Leu-2 (or T8)^{1,2} antigen is normally expressed on T lymphocytes that have cytotoxic or suppressor functions. Cells of a Leu-2 transfected clone were stained with fluorescein-tagged monoclonal anti-Leu-2, and the brightest 0.1–0.3% of cells were viably separated using a fluorescence activated cell sorter (FACS). After growth of these selected cells, sorting and regrowth was repeated six times, resulting in a population of cells that, compared with the starting population, stains 40 times brighter for Leu-2 and whose DNA transforms 20 times more efficiently for Leu-2. In addition, these cells have 10- to 50-fold amplified human DNA sequences and numerous double minute chromosome fragments, a common indicator of gene amplification in mouse cells.

Following co-transfection of mouse L cells (TK⁻) with the herpes simplex thymidine kinase (TK) gene and total human DNA from JM, a human T lymphoma line, we selected Leu-2⁺ and other human lymphocyte membrane antigen transferents by FACS sorting³. We found the frequency of transferents for Leu-2 to be about 10⁻³ of the TK⁺ cells selected in hypoxanthine-aminopterin-thymidine (HAT) medium. Although most cloned transferents had narrow ranges of antigen density per cell, one of the first four Leu-2⁺ transferents found was strikingly more variable in the amount of Leu-2 antigen per cell. Further, this transferent, J10, had a mean Leu-2 staining per cell that was seven times greater than that of the other transferents³.

To investigate whether the increased Leu-2 expression on the transfected cells was due to gene amplification, we sorted the brightest 0.3% of the cells aseptically with the FACS. These cells were grown, reanalysed, and the brightest cells again sorted and regrown. Each round took about 3 weeks. After six rounds of selection the mean fluorescence of the cells was about 40 times greater than that of the original cloned transferent J10 (Fig. 1); the mean brightness approximately doubled with each sort. Curiously, we found no further increase in mean fluorescence after more rounds of sorting. Further increases in Leu-2 expression may not have been possible because the surface was saturated with Leu-2 (The amount of Leu-2 antigen per cell matches the levels of H-2 antigen on these cells.) Increased Leu-2 expression was specific to Leu-2 since the amount of H-2 per cell remained unchanged throughout the sorting selections.

The presence of double minute (DM) chromosomes is characteristic of mouse cells that have amplified sequences^{4,5}. DMs are self-replicating acentric chromosomal fragments that are distributed unequally in daughter cells at mitosis. We examined metaphase spreads of J10-6 cells (the J10 transferent after six cycles of sorting) and could clearly see DMs in most spreads (Fig. 2). Variation from <10 to >100 in the number of DMs per spread was noted, which is like other counts of DMs in amplified mouse cell lines⁵.

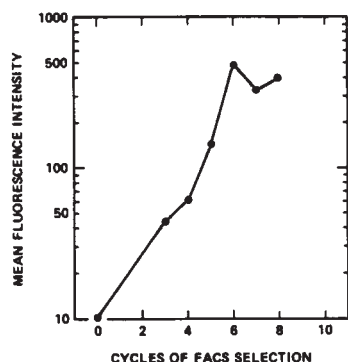


Fig. 1 Mean fluorescence of Leu-2⁺ transferent, J10, after each round of sorting the brightest cells. Starting with a cloned transferent J10, the cells were stained with fluorescein-conjugated anti-Leu-2a monoclonal antibody and the brightest 0.3% of the cells were sorted aseptically by the FACS. The cells were regrown, stained, analysed and resorted as described elsewhere except that propidium iodide was not used. The amount of anti-Leu-2a was sufficient for maximum staining. The mean fluorescence is calculated as the geometric mean of the distribution and expressed relative to 1.83 μ m green fluorescent beads (Polysciences Inc.) which were used as a standard of 100 fluorescence units. As the fluorescence of the cells increased the voltage of the photomultiplier was reduced or a neutral density filter was inserted.

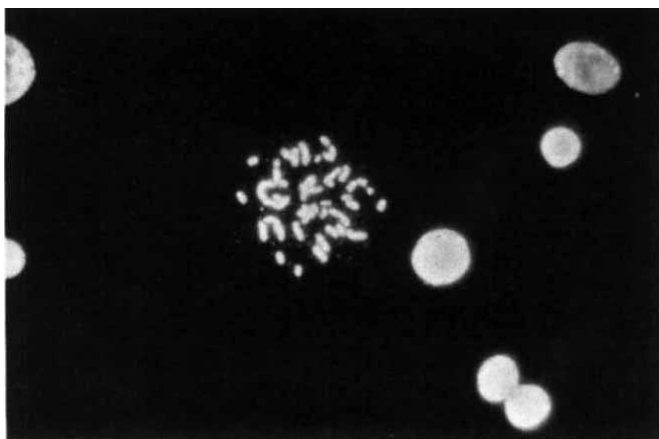


Fig. 2 Metaphase spread of J10-6 cells obtained after six cycles of FACS selection of sorting cells with increased antigen density. Metaphase spreads were stained with Hoechst 33258 stain (1 μ g ml⁻¹ in phosphate-buffered saline).

Because a Leu-2 gene probe does not yet exist for quantitation of Leu-2 gene copies by molecular hybridization, we used a biological assay, quantitative transfection, to test whether the Leu-2 gene number had increased. We found the transfection frequency of Leu-2 into mouse L cells to be roughly 15–20 times higher per μ g of DNA using DNA from the amplified line J10-6, than the frequency using JM or J10 DNA (Table 1). We next titred J10-6 DNA in a transfection experiment with different amounts of carrier DNA. When 1 μ g of J10-6 DNA was used with 20 μ g carrier (L cell) DNA per dish, Leu-2⁺ transferents could be detected on every dish (four dishes per experiment). No Leu-2⁺ transferents were detected with 1 μ g of JM DNA with the same amount of carrier DNA. This indicated that the Leu-2 gene was amplified at least 20-fold.

Analyses of DNA in stable transferents after introduction of specific genes into cells have shown that physically unlinked DNA segments tend to be physically linked in the transferents⁶. We would therefore expect that Leu-2⁺ DNA, other human DNA sequences and TK sequences would be ligated together

Table 1 Transformation frequency of Leu-2 into mouse L cells

DNA donor cell	No. Leu-2 ⁺ Cells* per 10 ⁴ TK ⁺ cells per dish
JM (T-cell lymphoma)	6, 17, 3, 6
J10 (Leu-2 transformant)	9, 10
J10-6 (J10 cells after six cycles of sorting)	97, 120, 157

Each dish was seeded with 10⁶ cells and overlaid with 20 μ g total DNA and 1 μ g of pBR322 plasmid containing the TK gene. Selection of transferents was as described previously³.

* Number of cells with fluorescence intensity above background. Background was fluorescence intensity of cells stained with second step antibody alone.

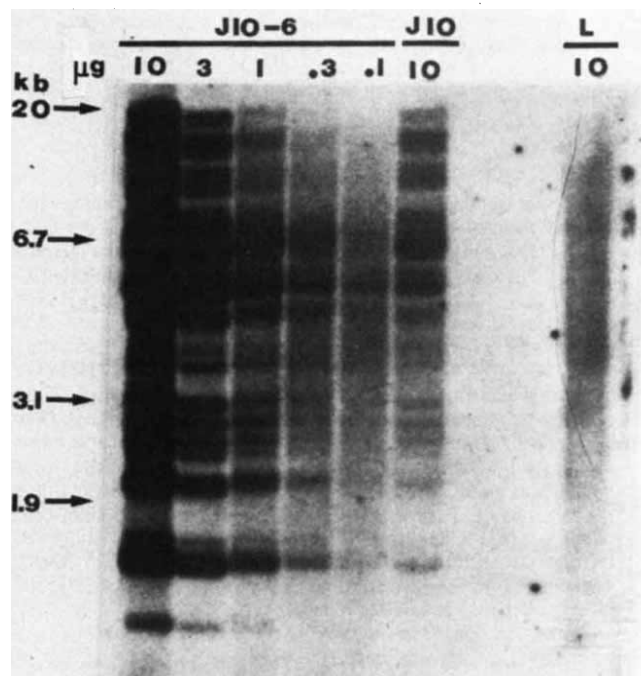


Fig. 3 Southern blot showing amplification of human repetitive sequences in cells with increased expression of Leu-2 on the surface. DNA was digested to completion with restriction endonuclease *Bam*HI, electrophoresed through a 0.75% agarose gel, blot transferred to a nitrocellulose filter, hybridized with ³²P-labelled human repetitive DNA¹⁰ (specific activity 2 × 10⁸ c.p.m. μ g⁻¹), and exposed to IAR-5 film with a Dupont Lightening Plus intensifying screen. J10 is a cloned Leu-2⁺ transferent of mouse L cells and J10-6 are J10 cells obtained after sorting the cells with highest antigen density six times. The amount of DNA (in μ g) analysed in each lane is indicated below the name of the cell line.

in transferents generated by co-transfection of mouse L cells with human DNA and the TK gene. We found indeed that all human DNA and many or all TK sequences were amplified in the J10-6 cells. Southern blots of DNA from J10, L cells and J10-6 were probed with human repetitive DNA (Fig. 3) or with the TK gene. Bands of the same size that were detected by human repetitive DNA probes in J10 were also present in J10-6 but with greatly increased intensity. We titred the amount of J10-6 DNA on the blot to see how much J10-6 DNA was required for the band intensities to be equal to those obtained with 10 μ g of J10 DNA and found that for most bands 0.2–0.3 μ g of J10-6 DNA was roughly equivalent to 10 μ g of J10 DNA; thus there was about a 30- to 50-fold increase in copy number of the *Alu* marked human sequences detected in J10-6. A few of the higher molecular weight bands, however, were amplified only about 10-fold. Southern blots with TK as a probe showed a similar 30- to 50-fold amplification (data not shown). Thus DNA sequences transfected into the L cells are amplified to approximately the same extent as the increase in Leu-2

expression on the cell surface. The sum of the sizes of the visible human repetitive DNA bands gives us an estimate of the amplified unit of at least 200 kilobase pairs.

Several pieces of evidence lead us to conclude that the structural gene for Leu-2 is amplified in the selected cells expressing high levels of Leu-2: (1) the transfection frequency for Leu-2 using J10-6 DNA is 15–20 times greater than with the same amount of donor JM lymphoma DNA or J10 DNA, (2) double minute chromosomes, known to occur with gene amplification in mouse cells, are present in J10-6 cells, (3) the introduced human or TK DNA integrated in the L-cell genome is amplified 10–50 times based on Southern blots with human repetitive DNA or TK as probes. Spontaneous amplification for the Leu-2 gene in a mouse L-cell transferent may be a fairly common event since at least 4 of 20 independent, FACS isolated, Leu-2 transformed clones showed an increased and unstable pattern of antigen expression. For each of the four clones we were able to select cells with greater antigen expression by FACS selection of the brightest stained cells. Preliminary work indicates that growing cells in HAT medium versus non-HAT medium is more effective for this process.

FACS has been used for selecting cells with other kinds of amplified sequences. Johnston *et al.*⁷ selected for amplification of dihydrofolate reductase (DHFR) in CHO cells by multiple cycles of sorting the brightest cells after staining with fluorescent methotrexate, an inhibitor of DHFR. This group obtained cells that had spontaneously amplified the DHFR gene 50-fold. With suitable fluorescent probes, selection for amplification of other genes may be made in a similar manner.

Cells with amplified copies of a gene coding for a cell-surface antigen could be very useful for cloning this gene. Cloning by cDNA methods could be simplified because the levels of mRNA are almost certainly increased in amplified cells. For example, Caskey *et al.*⁸ isolated a cDNA clone corresponding to the HPRT gene using a neuroblastoma line with 40-fold amplification. Double minutes could be isolated and used for the production of genomic libraries enriched for amplified sequences⁹. Cells with increased amounts of antigen expression should also make protein purification for structural and functional studies of cell-surface antigens easier.

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Immunoglobulin heavy chain binding protein

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Pre-B lymphocytes, and hybridomas derived from them, synthesize immunoglobulin heavy (IgH) chain in the absence of light (L) chain¹. In the Abelson virus transformed line 18-81, which is representative of the pre-B cell stage, we observed that at least some of the H-chains are bound to a protein other than L-chain. Here we show that the protein (which we term immunoglobulin heavy-chain binding protein, BiP) binds non-covalently to free IgH, but not to IgH associated with IgL.

Some of the 18-81 cells produce μ -chain and others produce γ 2b-chain. After fusion with a myeloma that does not synthesize any Ig chain, hybridomas can be recovered which produce μ - or γ 2b-chain, and some of which also produce κ -chain². Immunoprecipitation of intracellular Ig from the L-chain negative pre-B cell hybridomas resulted in the co-precipitation of a protein migrating with the same electrophoretic mobility as μ -chain in SDS-polyacrylamide gel electrophoresis (PAGE) in reducing conditions. We have previously shown that this protein of molecular weight 78,000 (78K) is not μ -chain². Figure 1a shows this protein from cells of the hybridoma line H6 which produces γ 2b chain but no L-chain (lane 2). In the μ -chain producing hybridoma H13, there is a similar protein, which can be distinguished from μ -chain, because this particular μ -chain migrates slightly faster than normal (Fig. 1a, lane 1). Very small amounts of the 78K protein also co-precipitated with the intracellular (Fig. 1a, lanes 3, 5) but not with the secreted immunoglobulin (lanes 4, 6) from the plasma cell hybridomas Sp2 and PC1 56.0, which produce IgG2b and IgG3, respectively. The 78K protein is not glycosylated (data not shown).

In order to establish whether the 78K protein that co-precipitates with intracellular immunoglobulin is the same in the different cell lines, peptide maps were analysed. The bands of interest were localized by autoradiography, cut out from the dried gels and subjected to limited proteolysis with papain³. In SDS-

PAGE, at least 10 peptides are shared among the 78K proteins from the γ 2b-producing hybridoma H6 (Fig. 1b, lanes 2, 6), from the μ -producing hybridoma H13 (lane 3) and from the plasma cell hybridomas Sp2 and PC1 56.0 (lanes 5 and 7 respectively). The peptide pattern of the γ 2b-chain (lane 1) differs from that of the μ -chain (lane 4) as well as from the peptide pattern of the 78K protein. From these results we conclude that the 78K protein is similar in the different cell lines.

The co-precipitation of the 78K protein with IgH chain could have been due to a cross-reactivity of the antisera used in these experiments. We have excluded this possibility by carrying out the precipitation with *Staphylococcus aureus* in the absence of any antiserum. *S. aureus* binds the Fc portion of mouse γ 2b-chain but not of μ -chain. The 78K protein does not bind to *S. aureus*; that is, no protein is precipitated in a cell lysate of μ -producing cells with *S. aureus* alone (Fig. 2, lane 3). However, on addition of anti- μ antiserum both the 78K protein and μ -chain are precipitated (Fig. 2, lane 5). Since the addition of *S. aureus* alone is sufficient to precipitate both the IgH chain and the 78K protein in γ 2b-synthesizing cells (Fig. 2, lane 6), the co-precipitation must be due to a binding of this protein to the γ 2b-chain. An explanation involving a binding of this protein to the *S. aureus* immunoglobulin complex can be excluded because (1) anti- κ antiserum together with *S. aureus* did not precipitate the 78K protein in the μ -producing hybridoma H13 (Fig. 2, lane 4); (2) the 78K protein was precipitated by anti-H chain antiserum together with a second antibody; (3) the precipitation of H chain and BiP was inhibited by the addition of unlabelled immunoglobulin of the same H-chain class, but not by the addition of immunoglobulin of other classes. Since the 78K protein thus binds to the γ 2b-chain, but also to the H-chain of other classes, we propose that this protein be called immunoglobulin heavy chain binding protein (BiP).

It is possible that the binding of BiP to H-chain is an artefact of the cell lysis and protein precipitation procedure—if BiP and H-chain were present in different cellular compartments the binding would occur only when the cells are lysed with detergent. To test this we analysed subcellular fractions obtained in the absence of detergent. BiP co-precipitated with H-chain in the microsomal fraction and in the soluble cytoplasm (Fig. 3a, lanes 2, 3, Fig. 3b, lanes 2, 3). Although the ratio of H-chain to BiP

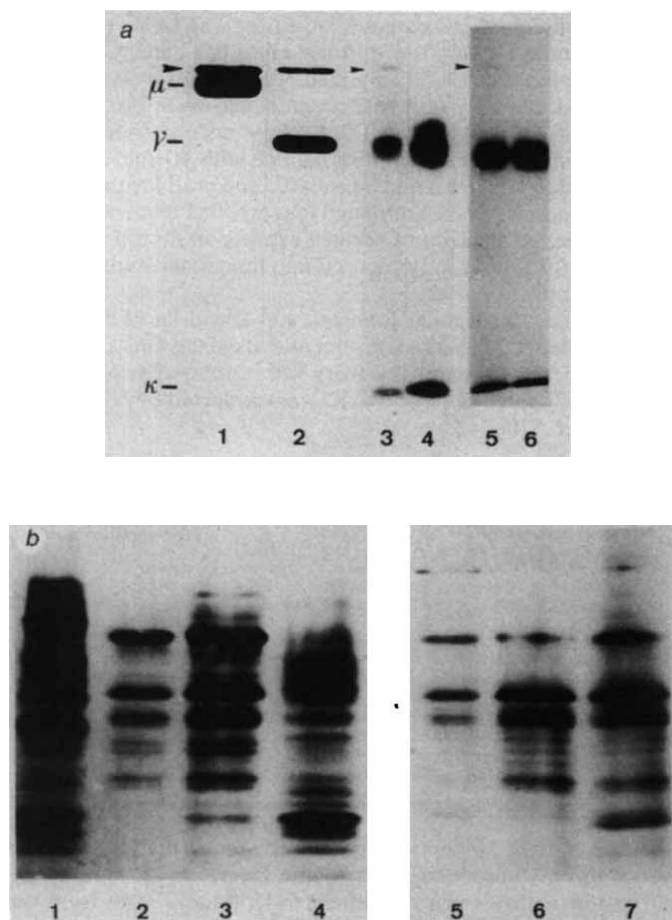


Fig. 1 A 78K protein that is co-precipitated with the intracellular immunoglobulin of various hybridomas. **a**, 2×10^6 cells were cultured in the presence of ^{35}S -methionine, lysed in 0.5% non-ionic detergent and the intracellular immunoglobulin was precipitated as described previously². Secreted immunoglobulin was precipitated from the labelled supernatant of the cells. Analysis of the precipitated material was performed by electrophoresis in 10% SDS-polyacrylamide gels. Lane 1, pre-B-cell hybridoma H13-synthesizing μ -chain precipitated with anti- μ ; lane 2, pre-B-cell hybridoma H6 synthesizing $\gamma 2b$ -chain precipitated with *S. aureus*; lanes 3, 4, plasma cell hybridoma Sp2 synthesizing IgG2b, intracellular (lane 3) and secreted (lane 4); lanes 5, 6, plasma cell hybridoma PCI 56.0 synthesizing IgC3, intracellular (lane 5) and secreted (lane 6); lanes 3–6, precipitation by *S. aureus* alone. **b**, Limited proteolysis of proteins was performed as described in the text. The ^{35}S -methionine-containing peptides were separated in 20% SDS-polyacrylamide gels and detected by fluorography. $\gamma 2b$ -chain of H6 (lane 1), 78K protein of H6 (lanes 2, 3), 78K protein of H13 (lane 4), μ -chain of H13 (lane 5), 78K protein of Sp2 (lane 6), 78K protein of PCI 56.0 (lane 7).

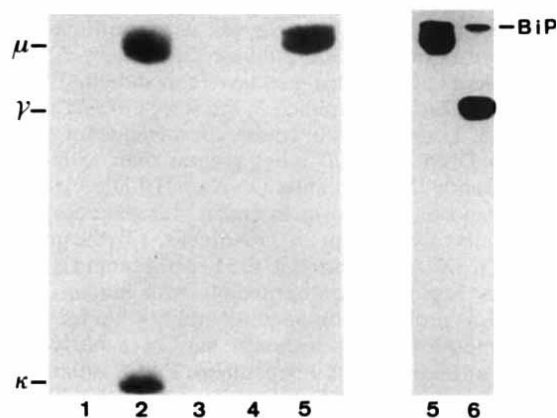


Fig. 2 The 78K protein binds to IgH chain. Experimental procedure was as for Fig. 1a. Precipitation by *S. aureus* was without antiserum in H11, synthesizing μ , κ (lane 1), in H13 (lane 3), and H33-11-2 (lane 6); or by *S. aureus* with antiserum to κ -chain in H11 (lane 2), in H13 (lane 4) or with antiserum to μ -chain in H13 (lane 5).

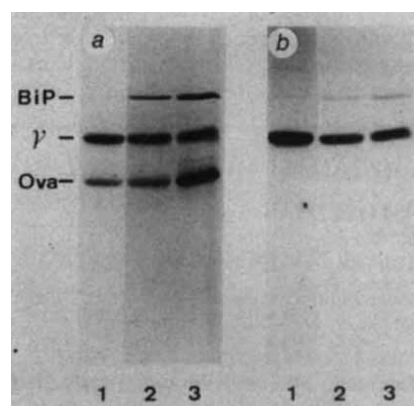


Fig. 3 Recovery of H-chain and BiP in the same subcellular compartments. **a**, Subcellular fractionation of $\gamma 2b$ -chain synthesizing pre-B-cell hybridoma H33-2 was performed as follows: 2×10^6 ^{35}S -methionine labelled cells were added to 10^8 non-labelled cells, and washed in 0.25 M sucrose. Lysis and immunoglobulin precipitation were performed as described in Fig. 1 with 1/10 of these cells (lane 1). The rest of the cells were homogenized in 1.5 ml sucrose, layered on 2 ml 0.34 M sucrose, centrifuged for 10 min at 600g to remove the nuclei and unbroken cells. After removal of the mitochondrial fraction (10 min at 5,000g), the soluble cytoplasmic proteins were separated from the microsomal fraction by centrifugation at 54,000g for 1 h. The pellet of the microsomal fraction was solubilized with non-ionic detergent (0.5% Triton X-100). Precipitation was performed by addition of *S. aureus* to the microsomal fraction (lane 2) and to the soluble cytoplasmic proteins (lane 3). The cytoplasmic proteins thus never came in contact with detergent. The gel was stained with Coomassie blue; Ova: ovalbumin. **b**, Autoradiography of the dried gel shown in **a**.

varies greatly in different precipitations from whole cell lysates (high in Fig. 3a, b, lane 1, lower in Fig. 1), in the cytoplasm and microsomal fraction the ratio of H:BiP is approximately 1:1, at least when comparing Coomassie blue staining intensity (Fig. 3a, lanes 2, 3). BiP incorporates less ^{35}S -labelled methionine than the $\gamma 2b$ -chain, which results in a higher H:BiP ratio after autoradiography of the same gel (Fig. 3b). Thus BiP is bound to H-chain when there is no detergent, and each fractionation step caused increased binding of BiP to H-chain. This suggests to us that BiP is associated with H-chain *in situ*, but this point remains to be proven formally.

BiP is bound noncovalently to H-chain in cells of the pre-B-cell line. Its expression in the pre-B-cell hybridoma is not induced by the fusion event. These conclusions are drawn from SDS-PAGE analysis in non-reducing conditions (Fig. 4a). The intracellular μ -chains of a subclone of the 18-81 cell line migrate

mainly as dimers (lane 3; the broadness of this band is presumably due to varying degrees of glycosylation of the μ -chains). Some of the μ -chains migrate as monomers. BiP migrates differently. It is resolved into two bands with a difference in apparent molecular weight of $\sim 1,000$. The same gel pattern is obtained with the μ -producing pre-B-cell hybridoma H13 (lane 2). BiP is also resolved into the same two bands in a $\gamma 2b$ -producing pre-B-cell hybridoma (lane 4). Both of these bands give rise to a 78K protein when rerun in reducing conditions (Fig. 4b). All other bands contain only $\gamma 2b$ -chain.

When the cells of the 18-81 cell line are fused with a myeloma which does not synthesize any immunoglobulin chain, some

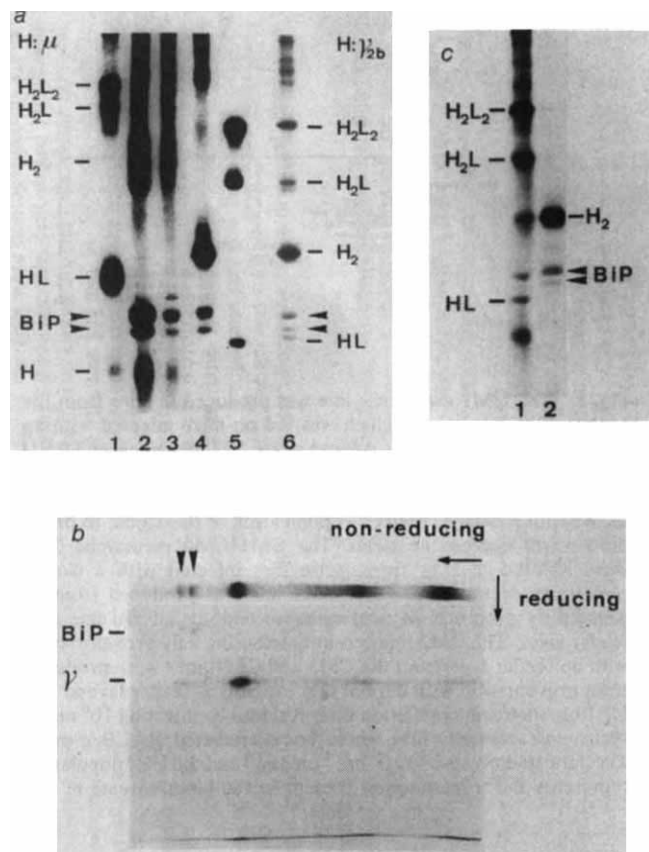


Fig. 4 Noncovalent binding of BiP to IgH-chain in pre-B cells and in hybridomas, but only when H-chain is not associated with L-chain. **a**, Cell labelling and immunoprecipitation as in Fig. 1. Immunoglobulin precipitates were analysed in non-reducing conditions in 5% SDS-polyacrylamide gels: H11, synthesizing μ , κ (lane 1), H13, synthesizing μ -chain (lane 2), 18-81 A33, synthesizing μ -chain (lane 3), H32-2, synthesizing γ 2b-chain (lane 4), H32, synthesizing γ 2b- κ (lane 5), H33-11-2, synthesizing μ , γ 2b and κ (lane 6, precipitation of μ not included). **b**, A γ 2b-BiP complex was first analysed in non-reducing conditions as described in **a**. Lane 4 of **a** was cut out from the dried gel, layered on top of a 10% SDS-polyacrylamide gel and rerun in reducing conditions. The γ 2b H-chain is found to smear throughout the lane of the nonreducing gel. **c**, Analysis of consecutively precipitated immunoglobulin from H32-1, synthesizing γ 2b- κ , in non-reducing conditions. Immunoprecipitation was performed with anti- κ antiserum coupled to Sepharose 4B (lane 1), the remaining immunoglobulin was precipitated by the addition of *S. aureus* (lane 2).

the fact is that the binding of BiP to H-chain is abolished when κ -chain binds to H-chain; this suggests that BiP is involved in the regulation of immunoglobulin chain synthesis—perhaps in the control of allelic and isotypic exclusion for immunoglobulin genes⁵.

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All metacyclic variable antigen types of *Trypanosoma congolense* identified using monoclonal antibodies

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Vaccination against the tsetse-borne trypanosomiasis has proved impossible because of the trypanosome's ability to generate a seemingly inexhaustible number of variable antigen types in the blood or tissues of the host¹. Each variable antigen is a glycoprotein which forms a surface coat on the trypanosome and each glycoprotein is the product of a single gene^{2,3}. The full repertoire of such antigens has not been identified for any trypanosome serodeme (genotype) as yet, but the number of genes coding for variable antigen glycoproteins is estimated to be between 100 and 1,000^{4,5}. We have previously postulated that for *Trypanosoma brucei* the antigen repertoire of the infective metacyclic stage trypanosomes inoculated by the tsetse fly may be considerably smaller than that expressed in the mammalian host⁶⁻⁸. If this is so then protection against infection by the vector becomes an easier proposition, but the actual scale of the metacyclic repertoire is also unknown. We present here evidence that the metacyclic repertoire of a stock of *T. congolense*, the most important of the pathogenic cattle trypanosomes, is limited to 12 variable antigen types.

T. congolense infected tsetse flies produce very few metacyclic trypanosomes and this inhibits examination of metacyclic variable antigen type (M-VAT) heterogeneity using fly-derived metacyclics. Recently, however, a culture system for generating *T. congolense* metacyclics *in vitro* has been developed⁹. Cultures are initiated with trypanosomes isolated from either the proboscis or proventriculus of infected *Glossina morsitans* and 2×10^6 metacyclics can be harvested daily from the supernatant of a mature culture; metacyclics are separated from their uncoated epimastigote predecessors by ion exchange chromatography¹⁰. We have utilized such metacyclics obtained from an uncloned West African stock, TREU 1290, to produce monoclonal antibodies against M-VATs. BALB/c mice were infected using 10^6 live culture metacyclics (UM1 population, Fig. 1). The mice were drug treated on day 5 with 10 mg per kg Berenil and injected again intravenously with 10^6 metacyclics on day 14. Spleen cells were removed 3 days later and fused with the myeloma cell line P3-X63-Ag8-653^{11,12}.

Twenty-five monoclonal antibodies (mAbs) were isolated and these labelled various subpopulations (VATs), of TREU 1290 metacyclics (UM1) using the indirect immunofluorescence technique on acetone-fixed trypanosomes. Twenty of these mAbs labelled exposed epitopes on live metacyclics. The percentage of the population labelled by individual mAbs varied from day

hybridomas can be recovered that produce κ -chain. In these hybridomas there is almost no BiP bound to H-chain (Fig. 4a, lane 1, shows a hybridoma producing $\mu\kappa$, and lane 5 shows a hybridoma synthesizing γ 2b- κ), although the H-chain is identical to the μ - or γ 2b-chain⁴ associated with BiP in the absence of κ -chain. Two-dimensional gel analysis of total cellular proteins revealed that BiP is still present in these κ -producing cells (data not shown). In hybridoma H33-11-2, not all of the IgH chains are associated with L-chains, and BiP is still synthesized and is co-precipitated in this κ -producing cell (Fig. 4, lane 6). Precipitation with anti- κ antiserum coupled to Sepharose 4B shows the occurrence of mixed molecules (H+H+L+BiP) (Fig. 4c, lane 1). Subsequent precipitation with *S. aureus* alone recovered H-chain dimers associated with BiP (Fig. 4c, lane 2). These findings can be explained in one of the following ways: (1) The binding of BiP might be prevented through steric hindrance by κ -chain; (2) a configuration change of the H-chain might occur when it is covalently bound to L-chain so that the binding site for BiP is altered; (3) BiP and L-chain might compete for the same binding site. Whatever the explanation,

to day. The figures presented in Table 1 are the mean percentages calculated from a count of at least 500 trypanosomes obtained over a period of at least 5 days for each mAb, and from a minimum of 2,000 per population for the pool of mAbs. Individually the mAbs labelled from <1% to 43% of the population; when pooled the 20 mAbs labelled 100% of the population. To determine how many VATs were labelled by the 20 mAbs, an indirect immunofluorescence assay was performed which involved adding all but one of the 20 mAbs required for total labelling, and then screening for less than 100% labelling. In this way it was found that 10 of the mAbs labelled unique VATs which none of the other mAbs labelled. Together as a pool, these mAbs, designated GUPMs 12.1–12.10, labelled 82% of the metacyclic population; the arithmetic total of the percentage labelling of GUPM 12.1–12.10 applied individually was greater than 100, however this anomaly may have arisen from the sampling of cultures for different M-VATs on different occasions, from the presence of cross-reacting epitopes on different VATs or from the presence of more than one VAT on individual trypanosomes. When individual mAbs were added to this pool it was found that upon addition of mAbs GUPM 12.11 and GUPM 12.12 the entire *in vitro*-derived metacyclic population from the uncloned stock could be labelled on any one day (Table 1).

The twelve mAbs GUPM 12.1–12.12 were then applied to metacyclic populations derived from a cloned population of the West African stock, to determine whether different metacyclic isolation protocols, or time in culture, affected the M-VAT repertoire. Regardless of the culture conditions used and the source of the culture, all metacyclic populations CM1–CM4 (Fig. 1) could be labelled entirely by the 12 mAbs, as could the few metacyclics obtained directly from infected tsetse flies (CFM populations), although the percentage of metacyclics labelled by a particular mAb varied from one population to another. What determines the proportion of M-VATs in a metacyclic population is as yet unknown. The UM1 metacyclic line was maintained *in vitro* for a period of 6 weeks, during which time the M-VAT repertoire remained constant even though the percentages of individual VATs present on different occasions varied.

Neutralization of infectivity of metacyclics for mice could be achieved by applying 500 μ l of a pooled cocktail of the 12 mAbs to 10^6 metacyclics in 500 μ l fresh guinea pig serum at 22 °C for 130 min and, after washing the trypanosomes in minimum essential medium plus 20% fetal calf serum, repeating the incubation with GUPM 12.3 and 12.10 before inoculating the trypanosomes into whole body irradiated (600 rad) BALB/c mice;

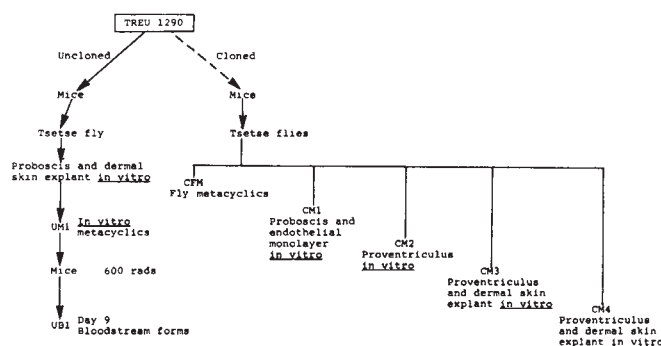


Fig. 1 The UM1 metacyclic line was produced *in vitro* from the proboscis of a tsetse fly which was fed on mice infected with an uncloned (prefix U-) West African stock of *T. congolense* (TREU 1290; Trypanosomiasis Research, Edinburgh University)⁹. Tsetse fly metacyclics (CFM) were obtained by allowing tsetse flies, infected with a cloned (prefix C-) population of this stock, to probe onto warm microscope slides. The CM1–CM4 metacyclic lines were isolated *in vitro* from tsetse flies infected with a cloned population of this stock. The CM1 line was produced from an isolated fly proboscis *in vitro* using an endothelial cell line as a feeder layer. The CM2 line was initiated using a fly proventriculus with no feeder layer, and the CM3 and CM4 lines were produced from proventriculi with dermal skin explants as feeder layers. The UB1 bloodstream population was produced by injecting 10^6 metacyclics intravenously into whole body-irradiated BALB/c mice. The parasitaemia rose to 10^9 ml⁻¹ on day 9 and the UB1 population represents the trypanosomes present in the blood-stream at this time.

omission of further treatment with the last two mAbs resulted in breakthrough of their respective VATs; presumably antibody titres in the cocktail were inadequate for effective neutralization of these two VATs. Removal of one of the mAbs from the pool likewise resulted in breakthrough of the corresponding VAT and a parasitaemia of 10^6 trypanosomes per ml expressing that VAT by day 7–8 in infected mice. Mice injected with successfully neutralized trypanosomes were still aparasitaemic 25 days after injection. Thus both immunofluorescence and neutralization studies suggest that the M-VAT repertoire of this stock is limited to 12 VATs.

In experimental cyclically-transmitted *T. brucei* infections the M-VATs are the first to appear in the bloodstream of fly bitten mice¹³. A similar picture is seen in irradiated mice infected with *T. congolense* metacyclics (UB1 population, Fig. 1; Table 1).

Table 1 Percentage labelling of trypanosome populations with monoclonal antibodies

Trypanosome population	UM1	UM1	CM1	CM2	CM3	CM4	CFM	UB1
Hybridoma line	Ac. fix	living	Ac. fix	Ac. fix	Form. fix	Ac. fix	Ac. fix	Ac. fix
GUPM 12.1	26	6	15	4	8	26	$\frac{1}{20}$	3
GUPM 12.2	15	18	2	12	5	12	$\frac{3}{13}$	26
GUPM 12.3	26	37	18	20	20	6	$\frac{1}{20}$	15
GUPM 12.4	10	5	2	12	20	4	$\frac{4}{20}$	18
GUPM 12.5	32	31	21	32	15	12	$\frac{3}{7}$	48
GUPM 12.6	12	2	20	21	8	6	$\frac{3}{20}$	2
GUPM 12.7	3	15	<1	<1	NL	<1	$\frac{2}{46}$	<1
GUPM 12.8	14	<1	2	10	NL	7	$\frac{9}{15}$	18
GUPM 12.9	<1	<1	NL	<1	NL	<1	$\frac{5}{30}$	<1
GUPM 12.10	45	20	22	30	31	52	$\frac{1}{13}$	26
GUPM 12.11	17	18	34	22	25	50	$\frac{4}{18}$	33
GUPM 12.12	20	8	10	18	10	10	$\frac{1}{17}$	8
Pooled GUPM 12.1–12.12	100	100	100	100	100	100	$\frac{312}{312}$	100

All populations were acetone fixed (Ac. fix.) except trypanosomes from the CM3 metacyclic line which were formalin fixed (Form. fix.). Living metacyclics and an acetone-fixed preparation of the UM1 population were used. Results obtained for the small number of metacyclics extruded by infected tsetse flies (CFM) are presented as the number of metacyclics labelled by the mAb over the number counted. For all other populations the percentage labelling was calculated on a count of at least 500 trypanosomes for each mAb, and a minimum of 2,000 per population for the pool of mAbs. (GUPM, Glasgow University Protozoology Monoclonal Antibody; NL, no labelling.)

The *T. congolense* M-VATs, moreover, appear to be more stable in the blood of irradiated mice; thus by day 9 a parasitaemia of 10^9 trypanosomes per ml was obtained, with the bloodstream trypanosomes expressing M-VATs in similar percentages to the metacyclic population. The entire bloodstream trypanosome population from these mice could be labelled using the 12 mAbs required for metacyclic repertoire labelling. This contrasts with the findings in immunosuppressed mice infected with *T. brucei* metacyclics where M-VATs are lost from the population by day 7 with non-metacyclic VATs appearing at day 4 post fly bite¹³.

The limited number of M-VATs present in this stock of *T. congolense*, and the relative ease with which large numbers of metacyclics and bloodstream forms expressing these VATs can be obtained, make this *in vitro* system an ideal model with which to study the gene rearrangements that occur during transformation from epimastigote to metacyclic and from metacyclic to early bloodstream form in African trypanosomes.

None of the mAbs which labelled metacyclics of this West African stock labelled metacyclics of an East African stock representing a different serodeme, although one mAb, GUPM 12.13, labelled the uncoated living epimastigotes of both stocks. The number of serodemes of *T. congolense* in the tsetse belt of Africa is unknown. For *T. brucei* subspecies infective to man, the number of serodemes circulating appears to be restricted. If *T. congolense* also exists in a limited number of serodemes, then vaccination of cattle against this form of tsetse-borne trypanosomiasis using a cocktail of M-VATs should be feasible.

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Note added in proof: The practical use of M-VAT based cocktail vaccines may be limited by the instability of the M-VAT repertoire. In a further paper¹⁴ we present data suggesting a rapid rate of change of M-VAT repertoire in a *T. brucei rhodesiense* serodeme during repeated cyclical transmission in the field and in the laboratory.

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Tripartite structure of the avian erythroblastosis virus E26 transforming gene

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Only two avian oncogenic viruses specifically cause acute leukaemias yet do not transform chicken fibroblasts in culture: E26, which causes erythroblastosis and a low level of concomitant myeloblastosis in chickens, and avian myeloblastosis virus (AMV), which causes myeloblastosis exclusively^{1–4}. Both viruses are replication-defective and share a sequence termed *myb* (also known as *amv*) which is unrelated to essential virion genes and is therefore thought to be part of the transforming *onc* genes of these viruses^{5,6}. However, the genetic structure of the two viruses differs⁷. E26 has a genomic RNA of 5.7 kilobases (kb) and encodes a 135,000 molecular weight *gag*-related protein (p135) with probable transforming function. We show here by *in vitro* translation that the 5.7-kb E26 RNA directs the synthesis of p135. Oligonucleotide analysis indicates that E26 RNA contains an internal 0.8-kb subset of the 1.2-kb AMV-related sequence (*myb*^A), termed *myb*^E. A 2.46-kb molecular clone prepared from cDNA transcribed *in vitro* from E26 RNA contained an E26 transformation-specific (*ets*) sequence flanked by *myb*^E and an *env*-related sequence. A complete DNA sequence of this clone indicates that the 1.5-kb *ets* sequence extends the open reading frame of *myb*^E for 491 amino acids. Thus, the p135 gene of E26 is a genetic hybrid of three distinct elements, ~1.2 kb derived from the 5' region of the retroviral *gag* gene, *myb*^E and the *ets* sequence, linked in the order 5'- Δ *gag*-*myb*^E-*ets*-3'. The myeloid leukaemogenicity shared by E26 and AMV correlates with the common *myb* sequence, while the distinct erythroid leukaemogenicity of E26 correlates with *ets* and the E26-specific linkage of *myb* to Δ *gag*.

To determine that genomic 5.7-kb E26 RNA, and not a spliced RNA, functions as mRNA for p135, the 60–70S E26 (E26-associated virus (E26AV)) RNA was heat-dissociated, poly(A)-selected and size-fractionated by sucrose gradient sedimentation. RNA from each gradient fraction was translated *in vitro* and the resulting ³⁵S-labelled protein was analysed by electrophoresis (Fig 1C). p135 was synthesized from 5.7-kb (31S) E26 RNA. The translation product is antigenically related to *gag* (not shown). This directly confirms our previous assumption that the 5.7-kb E26 virus RNA functions as mRNA for the E26-specific p135 protein⁷.

To characterize the *myb*^A-related sequences of E26 further, E26 RNA was hybridized to *myb*^A DNA (see Fig. 2). One experiment used AMV-specific cDNA that includes all *myb*^A sequences (Fig. 2A) while another used *myb*^A minus-strand DNA, which represents *myb*^A from DNA sequence position 289–1,309 (ref. 8) and which was molecularly cloned in phage m13 (Fig. 2B). E26 ³²P-RNA was isolated from a transformed myeloblast culture producing virus with a molar ratio of E26 RNA to E26AV RNA of approximately 1:1 and which also expressed p135 at a relatively high level compared with the helper virus proteins Pr180 and Pr76 (Fig. 1A,B). The E26 ³²P-RNA-AMV DNA hybrids were isolated, heat-dissociated and the RNase T1-resistant (T1) oligoribonucleotides were fingerprinted (Fig. 2A,B). The identity of the T1 oligonucleotides (Fig. 2) was confirmed on the basis of their RNase A-resistant fragments (data not shown; see refs 5, 8). A fingerprint of AMV RNA hybridized by *myb*^A-specific cDNA is shown for direct comparison (Fig. 2C, from ref. 5). *myb*^A is defined by 14 specific T1 oligonucleotides⁵, 101–114 (Fig. 2C), which map in the order 5'-102-101-105-104-107-106-108-113-109-110-112-111-103-114-3' according to the DNA sequence of AMV⁸. Seven of these and two newly analysed oligonucleotides, 115 and 116, are directly shared by E26 and AMV, and map in the order 5'-104-107-106-115-113-109-110-116-112-3' (see below, Fig. 3). T1 oligonucleotides corresponding to the AMV-specific oligonucleotides 101, 102, 103, 105, 108, 111 and 114 could not be detected in E26 RNA by these techniques. However, a sequence equivalent to AMV oligonucleotide 108 must also be present in E26, as E26 cDNA hybridizes oligonucleotide 108 of AMV⁵ (see Fig. 3, positions 241–259). The sequence positions of the *myb* T1 oligonucleotides clearly show that the shared E26 and AMV oligonucleotides form a

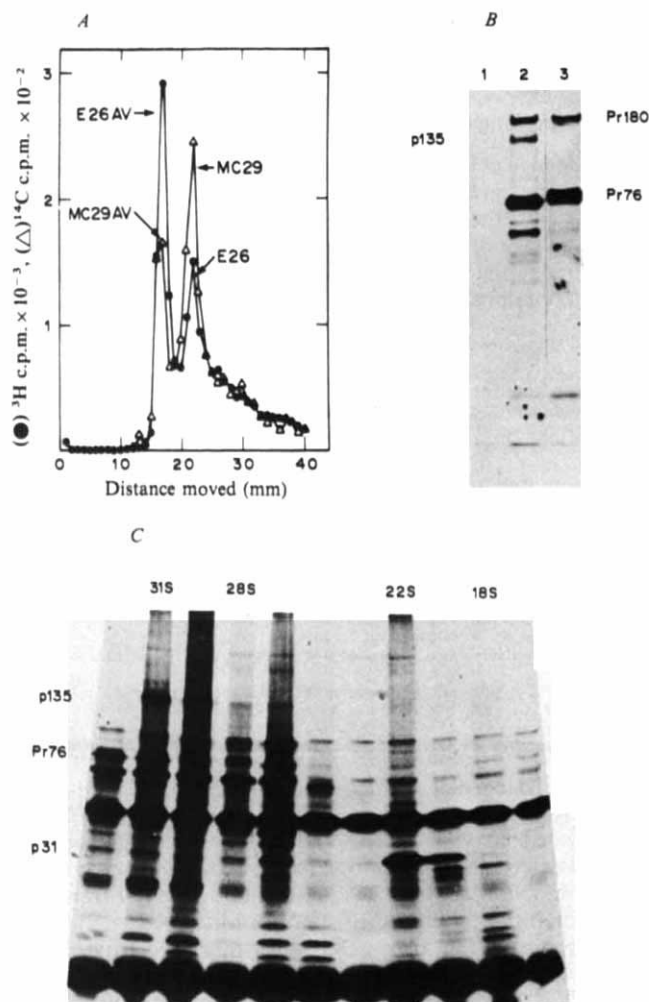
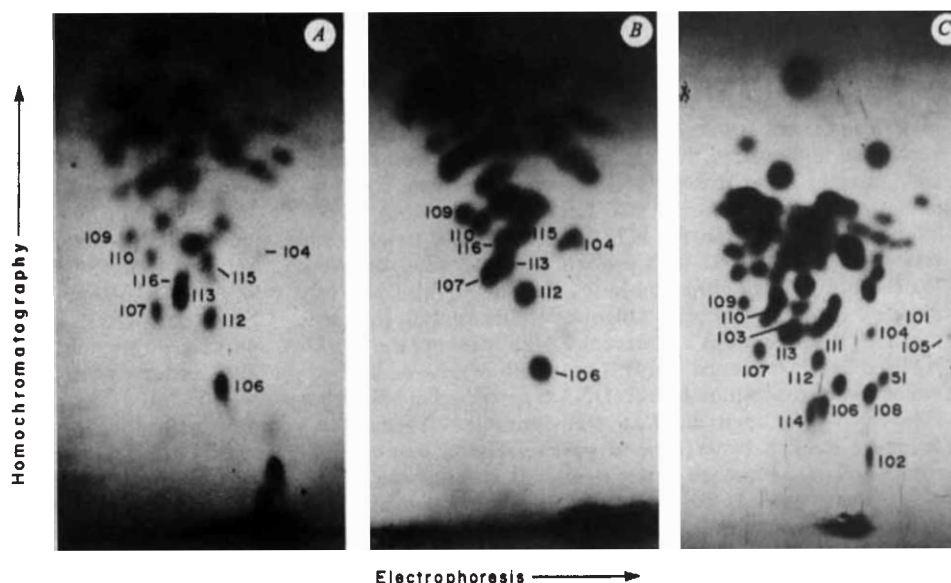


Fig. 1 Characterization of E26 RNA and its translation products. **A**, Electrophoresis of ^3H -RNA monomers of E26 (E26AV) in the presence of avian myelocytomatosis virus MC29 and MC29-associated virus (MCAV) ^{14}C -RNA standards^{7,16}. **B**, Analysis of E26-specific proteins. Immunoprecipitates of ^{35}S -proteins from quail myeloblasts transformed by E26 (E26AV) (lanes 1, 2) or the quail fibroblast line QT6¹⁷ infected with E26AV (lane 3) were prepared with rabbit preimmune serum (lane 1) or anti-disrupted MC29 virus serum (from T. Robins) (lanes 2, 3). **C**, *In vitro* translation of E26 RNA fractionated across a gradient. The positions of 28S and 18S ribosomal RNA markers sedimented in a parallel gradient are indicated.

Methods: **A**, E26 (E26AV) was injected into 1-day-old Japanese quail or chicken and transformed myeloblast cultures were isolated as described previously⁷. Cells were propagated in a growth medium of RPMI medium 1640 supplemented with 15% fetal calf serum, 5% heat-inactivated chick serum, 10% tryptose phosphate broth, and 100 $\mu\text{g ml}^{-1}$ folate. Preparation of ^3H - and ^{14}C -labelled 60–70S viral RNAs and electrophoresis in a 2.1% polyacrylamide gel followed published procedures¹⁸. **B**, Cells were labelled with ^{35}S -methionine and proteins extracted and immunoprecipitated as described elsewhere⁷. Electrophoresis in a SDS-7.5% polyacrylamide gel was carried out for 3 h at 60 V; the gel was fluorographed according to published procedures¹⁶. **C**, 2 μg E26 (E26AV) poly(A)-containing RNA was size-fractionated on a 15–25% sucrose gradient as described previously⁷. RNA from each fraction was translated *in vitro*, together with 5 μg yeast RNA carrier, in the presence of ^{35}S -methionine and the reaction products were analysed by electrophoresis in a SDS-6–18% gradient polyacrylamide gel and fluorographed as described previously¹⁹.

Fig. 2 RNase T1-resistant oligonucleotides of the *myb* regions of E26 and AMV RNA. E26 (E26AV) ^{32}P -RNA was annealed to AMV-specific cDNA (**A**) or m13 *myb* DNA (**B**) and the chromatographs of T1 oligonucleotides of the resultant RNase T1-resistant hybrids prepared according to published procedures⁵ are shown. A similarly treated hybrid of AMV (MAV) ^{32}P -RNA annealed to AMV-specific cDNA is shown (**C**). The compositions of the oligonucleotides detected here in E26 are given in Fig. 3 (with the exception of oligonucleotide No. 104, see text), and are reported in refs 5 and 8 for those identified previously in AMV.

Methods: AMV-specific cDNA was prepared as described previously⁵. 10^8 E26-producing, transformed myeloblasts were starved for 2 h in phosphate-free medium 199 supplemented with 10% dialysed calf serum, labelled for 4 h with 100 mCi carrier-free $\text{H}_3^{32}\text{PO}_4$ in 40 ml medium, and subsequently incubated with growth medium. $\text{H}_3^{32}\text{PO}_4$ uptake was ~40%, yielding upon purification of virus and isolation of viral 60–70S RNA¹⁸, 2×10^6 c.p.m. of RNA of unknown specific activity. Single-stranded m13 phage DNA complementary to *myb* was obtained from the *HaeIII*-*XbaI* fragment of AMV proviral DNA¹³ using m13 G ori 1 as a cloning vector, screened with AMV ^{32}P -RNA as probe (A. von Gabain, S. Cohen, M.F.N. and P.H.D., unpublished).



EcoRI PstI 50 100
 g aat tcc acc atg ogc cgg aag gtc gag cag gag ggt tac ctg cag gag tcc tcc aaa ggc cgt ooc tog gca acc acc ggc ttc cag aag agc ago caC ctg atg gcc ttt
 Asn Ser Thr Met Arg Arg Lys Val Glu Gln Glu Gly Tyr LEU Gln Glu Ser Ser Lys Ala Gly LEU Pro Ser Ala Thr Thr Gly Phe Gln Lys Ser Ser His Leu Met Ala Phe

. PstI 150 200
 gcc cac aac cca oot gca ggc cgc ctc cgg ggc gcc ggc cag gcc ccG ctg ggc agt gag tac ccc tac tac cac att got gag cca cca aat gtc oot ggt cag atc cca tat cca
 Ala His Asn Pro Pro Ala Gly Pro Leu Pro Gly Ala Gly Gln Ala Pro Leu Gly Ser Asp Tyr Pro Tyr Tyr His Ile Ala Glu Pro Gln Asn Val Pro Gly Gln Ile Pro Tyr Pro

. PvuIIPstI 250 300
 gta goa ctg eat Gta aat att Gtc aat gtt oot cag oca got got gca got att oag aga cac tat aAt gat gaa gac cct gag aaa gaa aag oga ata aag gaa tta gag ttg ota
 Val Ala Leu His VAL Asn Ile VAL Asn Val Pro Gln Pro Ala Ala Ala Ala Ile Gln Arg His Tyr ASN Asp Glu Asp Pro Glu Lys Glu Lys Arg Ile Lys Glu Leu Glu Leu Leu

350 SalI 400 450
 ctt atg tog act gag aat gaa ctg aaa ggc cag cag gca tta cca aca cag aac cac aca gca aac tac ccc ggc tgg cag ago acc aog gtt got gag aat acc aTg acc agt ggt
 Leu Met Ser Thr Glu Asn Glu Leu Lys Gly Gln Ala Leu Pro Thr Gln Asn His Thr Ala Asn Tyr Pro Gly Trp His Ser Thr Thr Val Ala Asp Asn Thr MET Thr Ser Gly

. 500 myb | ets 550
 gag aat goA cct gtt tcc tgt ttg ggc gaa cat cac cac tgt act cca tet cca cca gtc gat oat ggc acc toa gag atg atg agt tac tac atg gag aca acc att ggc agc agc
 Asp Asn Ala Pro Val Ser Cys Leu Gly Glu His His His Cys Thr Pro Ser Pro Pro Val Asp His Gly Thr Ser Glu Met Met Ser Tyr Tyr Met Asp Thr Thr Ile Gly Ser Thr

. PvuII 600 650BglI PstI
 ggt cct tat cct ttg gct cgc cct gga gtg atg caa ggt got ago ago tgc tgt gag gac ooc tgg atg cca tgc agg ctg cag tot gcc tgc tgc ooc ccc agg tog tgt tgc cgc
 Gly Pro Tyr Pro Leu Ala Arg Pro Gly Val Met Gln Gly Ala Ser Ser Cys Cys Glu Asp Pro Trp Met Pro Cys Arg Leu Gln Ser Ala Cys Cys Pro Pro Arg Ser Cys Cys Pro

700 750 HpaI 800
 ccc tgg gat gag ggc gcc atc cag gaa gtt ccc act ggc ctg gag cao tac agc aca gac atg gaa tgt goa gat gtg oot ttg tta acc ccc ago agc aag gaa atg atg tct cag
 Pro Trp Asp Glu Ala Ala Ile Gln Glu Val Pro Thr Gly Leu Glu His Tyr Ser Thr Asp Met Glu Cys Ala Asp Val Pro Leu Leu Thr Pro Ser Ser Lys Glu Met Met Ser Gln

. 850 900
 goa ctg aaa gcc acc ttc agt ggc ttc cca aag gag cag cag cgg ctg gga atc ccc aaa gat ooc cag cag tgg aca gag aoc cag ctg cgg gac tgg gtg atg tgg gca gtg aat
 Ala Leu Lys Ala Thr Phe Ser Gly Phe Ala Lys Glu Gln Gln Arg Leu Gly Ile Pro Lys Asp Pro Gln Gln Trp Thr Glu Thr His Val Arg Asp Trp Val Met Trp Ala Val Asn

. 950 1000
 gag ttc ago ctg aag gga gtg gat ttc cag aag ttc tgc atg aac gga got gcc ctg tgc gcc ooc aag gag tgc ttc ctg gag ota ggc cct gag ttt gtc gga gat atc oot
 Glu Phe Ser Leu Lys Gly Val Asp Phe Gln Lys Phe Cys Met Asn Gly Ala Ala Leu Cys Ala Leu Gly Lys Glu Cys Phe Leu Glu Leu Ala Pro Asp Phe Val Gly Asp Ile Leu

. 1100 1150
 tgg gaa cac ctg gag atc ttg cag aaa gaa gag gca aaa oca tac oca gaa aat gga gtg aat goa gog tat oca gaa tcc cgc tat act tea gac tac ttc att agt tat ggc atc
 Trp Glu His Leu Glu Ile Leu Gln Lys Glu Glu Ala Lys Pro Tyr Pro Ala Asn Gly Val Asn Ala Ala Tyr Pro Glu Ser Arg Tyr Thr Ser Asp Tyr Phe Ile Ser Tyr Gly Ile

. 1200 1250
 gag oac gca cag tgc gtg oot ccc tcc gag ttc tot gag ooc ago ttc atc aca gag tcc tac cag acc ctc cat ccc atc ago tgc gaa gag oot ctg tcc otc aag tac gag aac
 Glu His Ala Gln Cys Val Pro Pro Ser Glu Phe Ser Glu Pro Ser Phe Ile Thr Glu Ser Tyr Gln Thr Leu His Pro Ile Ser Ser Glu Glu Leu Leu Ser Leu Lys Tyr Glu Asn

. PstI 1300 1350
 gag tat ccc toa gtc atc oot cgt gag ccc gtc cag aoc gag tcc ctg cag aca gac tac ttc aca atc aag caa gaa gtg gta aoc cca gac aac atg tgc atg gga cgt gtc agt
 Asp Tyr Pro Ser Val Ile Leu Arg Asp Pro Val Gln Thr Asp Ser Leu Gln Thr Asp Tyr Phe Thr Ile Lys Gln Glu Val Val Thr Pro Asp Asn Met Cys Met Gly Arg Val Ser

. PvuII 1450 1500 PstI
 oga ggt aaa ctg ggt ggc cag gac too ttt gag agc ata gag ago tac gag agc tgt gag cgc ctg aca cag tcc tgg ago agc cag tcc tcc ttc cag agc ctg cag cgc gtc ooc
 Arg Gly Lys Leu Gly Gly Gln Asp Ser Phe Glu Ser Ile Glu Ser Tyr Asp Ser Cys Asp Arg Leu Thr Gln Ser Trp Ser Ser Gln Ser Ser Phe Gln Ser Leu Gln Arg Val Pro

. 1550 1600
 tcc tac gat agc ttt gag toa gag gac tac ooc gcc ccc ctg ooc aac cca aag ccc aag ggc acc ttc aag gac tat gtt oga gat agg got gag atg aac aag gac aag cct gtc
 Ser Tyr Asp Ser Phe Asp Ser Glu Asp Tyr Pro Ala Ala Leu Pro Asn His Lys Pro Lys Gly Thr Phe Lys Asp Tyr Val Arg Asp Arg Ala Asp Met Asn Lys Asp Lys Pro Val

. 1650 1700 PvuII
 att oot gcc got gcc oot gcc ggc tao aca ggc agt gga ooc ato caa ctg tgg caa ttc ctg ctg gag ctg ctc act gag aag tcc tgt cag tcc too ato ago tgg aoc ggt gat
 Ile Pro Ala Ala Ala Leu Ala Gly Tyr Thr Gly Ser Gly Pro Ile Gln Leu Trp Gln Phe Leu Leu Glu Leu Leu Thr Asp Lys Ser Cys Gln Ser Phe Ile Ser Trp Thr Gly Asp

. HindIII 1800 1850
 ggc tgg gag ttc aag ctt too gat oca gat gag gtg gcc agg ogg tgg ggc aag agg aaa aac aag ooc aag atg gac tat gag aag ctg ago cgt ggt ctg cgt tac tat tac gac
 Gly Trp Glu Phe Lys Leu Ser Asp Pro Asp Glu Val Ala Arg Arg Trp Gly Lys Arg Lys Asn Lys Pro Lys Met Asp Tyr Glu Lys Leu Ser Arg Gly Leu Arg Tyr Tyr Tyr Asp

. 1900 PstI BglI 1950
 aag aac gtc atc cac aag aoc gcc ggc aag cgc tac gtc tao ogo ttc gtc tgc gac ctg cag ago ctg ctg ggc tac aca cca gag gag aac toa toa gca tot ggc ttg aoc too
 Lys Asn Val Ile His Lys Thr Ala Gly Lys Arg Tyr Val Tyr Arg Phe Val Cys Asp Leu Gln Ser Leu Leu Glu Tyr Thr Pro Glu His Ser Ser Ala Ser Gly Leu Thr Ser

. 2000 ets | env 2050 2100
 agc atg ggc tgc agc too ttt tga oagacccaggaacatccttcttcttgataggccctcCaactccTcgAaacogttTacAgtAgtAaacagcgaCagGCacaatctCttatggggagGagtaTtgggtcatatggetacag
 Ser Met Ala Cys Ser Ser Phe ***

. 2150 2200 2250
 GttCtgggaaatGtaCaaTtgcTcaaaAaTgagACAGaatTGGTCoAtctgtCAGGaTgtTgGggCCGAggcoctTccGgaaGTTgtgtTaCaAgTaccagaggtatatgggttaatacaatoGaaggTaataCaaCgagacagaAccgttoagtt

. 2300 2350 SalI 2400
 ttactgcaactgtactggaatCtgggttaaCgtcagggatgttggtggagaaacGatcagcattctccacCagggccatggGtagacagtaoagaggaagttTactaaacaaaagcgtacacccgcgaattttctcatgttggg

. 2450 EcoRI
 gatcgogcatggcaGgaatttc

Fig. 3 DNA sequence of the 2.46-kb *EcoRI* fragment of E26 from the *EcoRI* to *SalI* restriction sites. The amino acids of the p135 protein deduced from the DNA sequence are given. The borders of *myb*, *ets* and *env* are shown. Base changes with respect to AMV⁸ or Pr-C RSV¹² and the corresponding differences in amino acids are indicated by capitals. The positions of *myb*^E RNase T1-oligonucleotides (beginning with no. 107 and continuing in the order mentioned in the text) are underlined and restriction endonuclease sites are indicated.

Methods: *SalI*, *HpaI* and *HindIII* fragments of purified 2.46-kb pER4 *EcoRI* insert were subcloned into phage m13 and sequenced by the incorporation of ³²P-labelled nucleoside triphosphates onto a synthetic primer in the presence of individual dideoxynucleoside triphosphates using the m13 DNA as template and analysis of the products on polyacrylamide gels as described previously²².

contiguous block of sequences in E26 RNA colinear with the nucleotide sequence positions 410–1,029 in AMV. As *myb*^A spans sequence positions 78–1,227⁸, this corresponds to the middle 0.8 kb of the *myb*^A sequence.

These results show that there is no E26 equivalent to the *myb*^A– Δ env 3' region at position 1,153–1,313 that encodes the carboxy terminus of the AMV p48 product^{8,9}. E26 also does not contain the sequences of *myb*^A which result in the termination of the p180 *gag*– Δ pol product of AMV and simultaneously function as the splice acceptor site for AMV mRNA^{8,10}. These

sequences map 5' to the boundary of overlap between AMV and E26 at *myb*^A positions 78–164⁸. Figure 4 shows the alignment of the *myb* regions in E26 and AMV.

As the *gag*-related hybrid *onc* gene of E26 is 3.6 kb long, based on the size of its p135 product, this gene must contain at least 1.1 kb of specific genetic elements in addition to the *gag*-related elements of 1.7 kb and the 0.8-kb *myb*^E sequence. The 1.7-kb is a maximum estimate of Δ *gag* based on our experience that the *gag* component of hybrid *onc* genes of several other avian retroviruses, with the generic Δ *gag*–x design all measure

1.3–1.7 kb (ref. 11) and on the fact that the *gag* complement must be incomplete (<2 kb), because 31S E26 RNA does not appear to direct the synthesis of a complete Pr76 *gag* protein *in vitro* (Fig. 1C) or in cells in the absence of helper virus⁷.

To identify the remaining E26-transforming gene specific sequence, E26 cDNA, transcribed *in vitro* from viral RNA by reverse transcriptase, was prepared and analysed after molecular cloning. Figure 5 shows the analysis of a plasmid DNA containing a 2.3-kb *Eco*RI-resistant E26 DNA fragment (termed pER4) by Southern blotting. The 2.3-kb *Eco*RI insert (Fig. 5a) hybridizes with both *myb* (Fig. 5c) and *env* (Fig. 5e) probes. *Hind*III cleaves this fragment into 0.7- and 1.6-kb fragments (Fig. 5b). The 1.6 kb-fragment hybridizes with the *myb* probe (Fig. 5d) while the 0.7-kb fragment hybridizes with the *env* probe (Fig. 5f), indicating that these sequences lie at opposite ends of the 2.3-kb clone.

Further analysis of the pER4 subclone using the restriction enzymes *Pst*I and *Pvu*II revealed E26-specific sequences between the terminal *myb* and Δenv sequences (Fig. 5g–l). *Pst*I cleaves the 2.3-kb fragment into 0.65, 0.42, 0.39 kb, and smaller DNA fragments (Fig. 5g). The 0.39-kb fragment hybridizes with the *myb* probe (Fig. 5i) and the 0.52-kb fragment hybridizes with Prague Rous sarcoma virus subgroup B (Pr-B RSV) ³²P-RNA (Fig. 5k). The 0.65- and 0.42-kb pieces hybridized neither the *myb* probe nor Pr-B-RSV RNA and represent E26-specific sequences. Similarly, *Pvu*II cleavage yields 0.79, 0.69, 0.37 and 0.28 kb DNA fragments (Fig. 5h) of which the 0.37-kb piece is *myb*-related (Fig. 5j), the 0.69-kb piece is related to Pr-B- RSV (Fig. 5l) and the 0.79-kb piece is E26-specific. These experiments and others have been used to generate the restriction map shown in Fig. 5. The 2.3-kb insert was not cut by *Bam*HI, *Cla*I, *Kpn*I, *Pvu*I, *Sst*I, *Xba*I or *Xho*I.

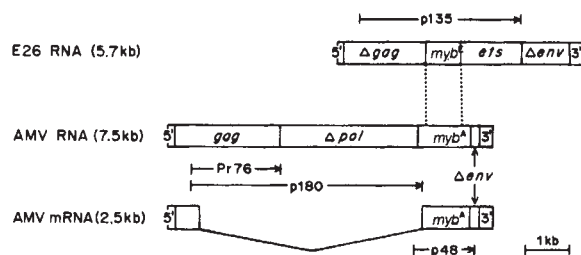
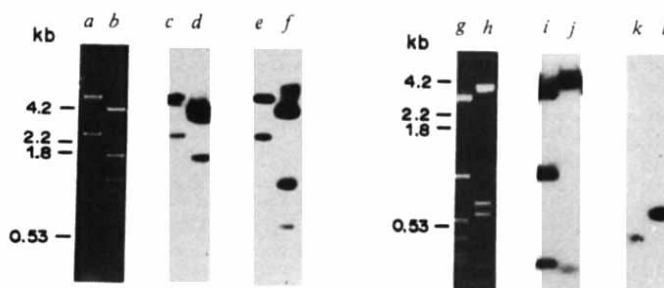


Fig. 4 The genetic structures and protein products of AMV and E26. The boundaries of the *myb* region shown correspond to sequence positions 78 and 1,277 in the AMV DNA sequence published by Rushlow *et al.*⁸.

In experiments not shown, hybridization of pER4 DNA with Pr-C RSV ³²P-RNA, analysis of the hybrid by RNA fingerprinting as described for Fig. 2 and comparison of the oligonucleotides obtained with the known DNA sequence of Pr-C RSV indicated that this E26 clone contains ~0.4 kb of *env* mapping 5' to the *Eco*RI site of Pr-C RSV (at position 6,144)¹². Also, the *myb*-containing *Sall*-*Eco*RI fragments of pER4 and the *myb*^A clone pHAX4¹³ are both 0.36 kb by agarose gel electrophoresis. This suggests that the 5' and 3' *Eco*RI sites of the pER4 2.3-kb insert correspond to the same sites in *myb*^A (at DNA sequence position 510)⁸ and in the *env* of Pr-C, respectively and that these sites represent original *Eco*RI sites in E26, rather than in DNA linkers used to prepare the clone (see Fig. 3). In addition, Southern blots of genomic DNA from E26-infected myeloblasts restricted with *Eco*RI yield a 2.3-kb fragment hybridizing to *myb* (M.F.N. and P.H.D., unpublished). Thus, cDNA cloning of E26 viral RNA has yielded an internal E26 *Eco*RI fragment which contains an E26-specific region bordered on its 5' side by *myb* sequences and on its 3' side by *env*-related sequences. We have termed this specific sequence *ets*.

Figure 3 gives the complete DNA sequence of this *Eco*RI fragment. The open reading frame of *myb*^E extends directly into the 1.5-kb *ets* sequence for 491 amino acids. As there are multiple stop codons in the two alternative reading frames of

the *myb*-*ets* sequence, it is unlikely that E26 codes for other specific proteins unrelated to p135. As predicted by the RNA fingerprinting analysis, the 3' portion of *myb*^A is missing from the E26 genome; however, the shared *myb* sequences are highly conserved, with 9 base differences out of 533 resulting in the 6 amino acid differences noted in Fig. 3. The *myb*-related RNase T1 oligonucleotides of Fig. 2, with the exception of oligonucleotide 104 which maps 5' to the *myb* *Eco*RI site (at AMV sequence position 400)⁸, are underlined in the E26 sequence, beginning



E26 2.3kb *Eco*RI fragment:



Fig. 5 Analysis of the E26 cDNA clone pER4 by restriction endonuclease digestion and hybridization with *gag*, *pol*, *env* and *myb* probes. In a, c and e, the plasmid is digested with *Eco*RI alone; in b, d and f, with *Eco*RI and *Hind*III; in g, i and k, with *Eco*RI and *Pst*I; and in h, j and l, with *Eco*RI and *Pvu*II. Lanes c, d, i and j show hybridization with *myb* DNA, lanes e and f with *env* DNA, and lanes k and l with Pr-B RSV RNA. A restriction endonuclease map of the 2.3-kb *Eco*RI E26 insert of pER4 is shown: E, *Eco*RI; H, *Hind*III; P, *Pst*I; PII, *Pvu*II; S, *Sall*.

Methods: Cloning of cDNA transcribed from E26 RNA was performed according to the methods of T. Huynh and R. Davis (personal communication). cDNA was synthesized from 2 µg heat-dissociated 60–70S E26 RNA using an oligo-dT primer and reverse transcriptase. The resulting cDNA was made double-stranded with bacterial DNA polymerase. The ends of the DNA were made blunt with *S*₁ nuclease, and synthetic *Eco*RI restriction sites were linked to the E26 DNA with T4 ligase. The DNA mixture was then digested with *Eco*RI and the high molecular weight species cloned into the *Eco*RI site of bacteriophage λgt10 DNA. Recombinant phage DNA was packaged *in vitro*, screened and purified according to established procedures²⁰. On screening with ³²P-labelled *myb* DNA, cloned in the plasmid pHAX4¹⁴, more than 200 *myb*-positive phage containing E26 sequences were obtained. Nine of these were subsequently analysed: three contained *Eco*RI inserts of about 8 kb and were not analysed further; six contained *Eco*RI inserts of 2–2.3 kb. The largest insert of this latter class was subsequently ligated into the single *Eco*RI site of the 4.9-kb plasmid pBR328 DNA (from M. Lusky²¹), transfected into *Escherichia coli* strain HB101 with CaCl₂ shock, and the cells were screened for chloramphenicol sensitivity²⁰. Plasmid DNAs were amplified and purified according to established procedures²⁰. Lanes a, b, g and h are photographs of ethidium bromide-stained 1.2% agarose gels after electrophoresis for 3 h at 100 V in Tris-acetate buffer, pH 8. Lanes c, d, i and j are autoradiographs of nitrocellulose filters blotted from the same gels according to the method of Southern and hybridized with the ³²P-labelled pHAX4 described above²⁰. Lanes e and f are from an autoradiograph of a filter prepared as in c and d, but hybridized with ³²P-labelled *env* DNA (pH *env* XB-1 from J. Wills and E. Hunter). Lanes g and h are an autoradiograph of the filter prepared from the same gel as in g and h, hybridized with ³²P-labelled Pr-B RSV RNA. ³²P-labelling of DNAs by nick translation, hybridization and washing of the filters were performed essentially as described previously⁷. Size markers shown are for the *Hind*III fragments of λ phage DNA (New England Biolabs). Restriction fragments not identified in the text are from the plasmid vector.

with oligonucleotide 107 at position 116 and ending with 112 at position 507. The *ets* sequence of the 2.45-kb *EcoRI* fragment is flanked at its 3' end by a portion of an *env* gene which shows several base differences from the *env* sequence of Pr-C RSV¹² (see Fig. 3).

It is likely that the *ets* sequence is derived from a cellular gene, proto-*ets*, distinct from proto-*myb*, because pER4 does not hybridize with two proto-*myb* clones (1,041 and 1,132) which extend 18 kb 5' and 14 kb 3' from proto-*myb* (ref. 14), and proto-*ets* clones do not hybridize with the *myb*^A probe pHAX4¹³ (not shown). The junction of *myb*^E and *ets* at sequence position 533 (Fig. 3) corresponds to *myb*^A sequence position 1,039⁸, which lies within an exon of proto-*myb* (ref. 10), indicating that a recombination between a coding sequence of proto-*myb* and the unlinked proto-*ets* locus was involved in the generation of E26.

In conclusion, we have shown that the 5.7-kb genomic E26 RNA functions as mRNA, that the *gag*-related p135 product defines 3.6 kb that is the probable transforming gene of E26, and that this hybrid gene includes an internal subset of the *myb* sequence of AMV of ~0.8 kb, termed *myb*^E, and an E26 transformation-specific coding sequence of 1.5 kb, termed *ets*. The *ets* sequence is unrelated to the *myb* sequence or to the *onc* genes of RSV and, as shown previously, of myelocytomatosis virus MC29 and avian erythroblastosis virus⁵. We have mapped the sequences of the E26 *onc* gene in the order Δgag -*myb*^E-*ets*. Consistent with our findings that p135 contains *myb*-related elements, p135 has recently been shown to share *myb*-specific determinants with the p48 protein encoded by *myb*^A (ref. 9).

Therefore, E26 and AMV represent a unique pair of retroviruses in which a common *onc* gene-specific sequence, *myb*, is expressed in two completely different ways. Each of these two structurally related genes may cause myeloblastosis via the common *myb* domain; however, it is also possible that myeloblastosis by E26 is induced by a distinct mechanism as has been suggested by biological studies of the virus²³. E26 is further unique in that its transforming gene is a complex tripartite hybrid containing sequences from the retroviral *gag* gene and two unlinked cellular genes, proto-*myb* and proto-*ets*. By contrast, the *onc* genes of other known retroviruses are thought to be derived from one cellular gene, or from one cellular gene and an essential virion gene^{11,15}. These three domains differ in function from the parent retroviral and probably the cellular genes from which they derive, combining to form the single oncogenic unit of E26.

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Note added in proof: We have recently cloned and analysed proviral E26 DNA. The partial sequence analysis of this clone agrees with the results described here and has determined the coding region of the Δgag element of E26 to be 0.8 kb. Hence the 3' *gag* terminus of E26 corresponds to sequence position 1196 of Pr-C RSV (ref. 12) and the 5' terminus of *myb*^E to sequence position 193 of AMV (ref. 8).

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A putative second cell-derived oncogene of the avian leukaemia retrovirus E26

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The acute avian leukaemia retroviruses AMV and E26 both induce myeloblastosis *in vivo* and transform myeloblasts *in vitro*^{1–5}. Both viruses contain the oncogene *v-myb* first described for AMV^{6,7}. Unlike AMV, E26 has the additional capacity to induce erythroblastosis *in vivo* and to transform erythroblasts^{4,5}. Previous analyses indicated that the genome of E26 also contained nucleotide sequences distinct from *v-myb* and unrelated to viral replicative genes^{6,8,9}. Using a molecularly cloned E26 provirus, we have now identified a novel nucleotide sequence designated *v-ets* (for *E*-twenty-six specific)¹⁰ of ~1.5 kilobase pairs (kbp) located next to *v-myb*. *v-ets* possesses all the structural characteristics of a putative new oncogene: it has a conserved cellular counterpart *c-ets* which is transcribed in some normal chicken cells as a major 7.5-kb polyadenylated RNA. Although our results now await elucidation of their biological significance, we propose that *v-ets* could be a new oncogene accounting for the additional transforming properties of E26, or potentiating the transforming properties of the *v-myb* oncogene.

A quail recombinant gene library was prepared in λ phage Charon 4A by partial *EcoRI* cleavage of DNA from an E26-transformed quail fibroblast cell clone (E26 QC1). The library was screened with a viral *myb* probe^{6,11} or probes of structural viral genes (*gag*, *pol*, *env*)^{1,12} or the long terminal repeat (LTR) of ASV¹³. A recombinant phage (λ E26 Q1) was shown to contain the E26 provirus (Fig. 1). A *HincII*-*SalI* fragment of 1.6 kbp (Fig. 1, fragment *a*) hybridized with none of the above probes, except faintly in its 3' end (*PstI*-*SalI* region) with the *env* probe (data not shown). This fragment thus contained E26-specific nucleotide sequences, denoted *v-ets* according to published nomenclature¹⁰, that were located downstream of the E26 *v-myb* sequences.

We then examined if *v-ets* had a normal cellular counterpart. The 1.6-kbp *HincII*-*SalI* fragment was used as a *v-ets* probe (probe *a*, Fig. 1). Normal chicken DNA digested with *EcoRI* hybridized with probe *a* as shown in Fig. 2, lane 2. Similar studies with quail and human DNA also exhibited several positive bands (Fig. 2, lanes 3 and 4, respectively). Therefore, *v-ets* had a cellular counterpart *c-ets* in all the vertebrate DNAs tested. This pattern was different from those described for the cellular oncogenes (*c-myb*, *c-erbA*, *c-erbB* and *c-myc*) corresponding to the other avian defective leukaemia viruses^{1,14–17}.

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λ E 26 Q1

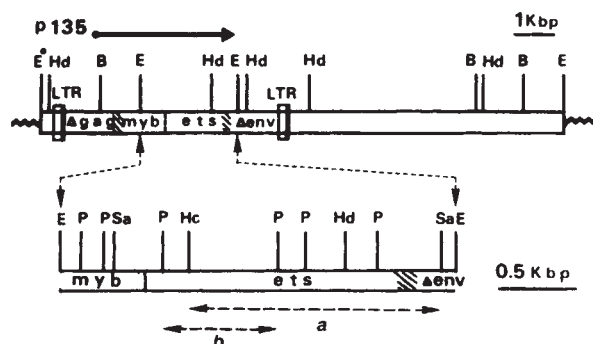


Fig. 1 Map of E26 molecularly cloned provirus. The clone λ E26 Q1, isolated as described in the text, was analysed by restriction endonuclease fragmentation followed by Southern blotting and hybridization with cDNA *myb*^{6,11}, and cloned probes representing viral genes (*gag*, *pol*, *env*)^{6,12} or the LTR of a cloned ASV DNA (SRA-2) as described in ref. 13. Restriction endonucleases were: B, *Bam*HI; E, *Eco*RI; Hd, *Hinc*II; Hd, *Hind*III; P, *Pst*I; Sa, *Sal*I. The *Eco*RI 2.5-kbp fragment contains an additional 0.07-kbp *Pst*I fragment not yet mapped. E, *Eco*RI site expected from the construction of the gene library but not found in this clone. Wavy lines, Charon 4A DNA. ⌘, the putative boundaries not precisely determined between *gag* and *myb* or between *env* and *ets* domains. <--->, Fragments used as *v-ets* probes in Figs 2 and 4. →, Indicates the fusion protein p135 known for this virus to initiate into the *gag* gene^{8,24}, which calculations indicate must extend through the *myb* sequences and terminate around the 3' end of the *ets* sequences.

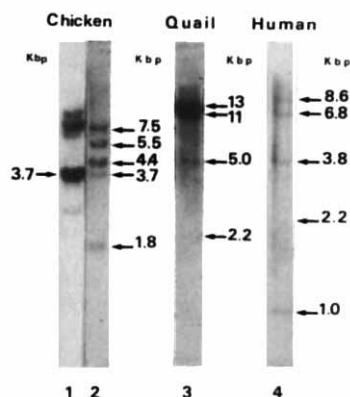
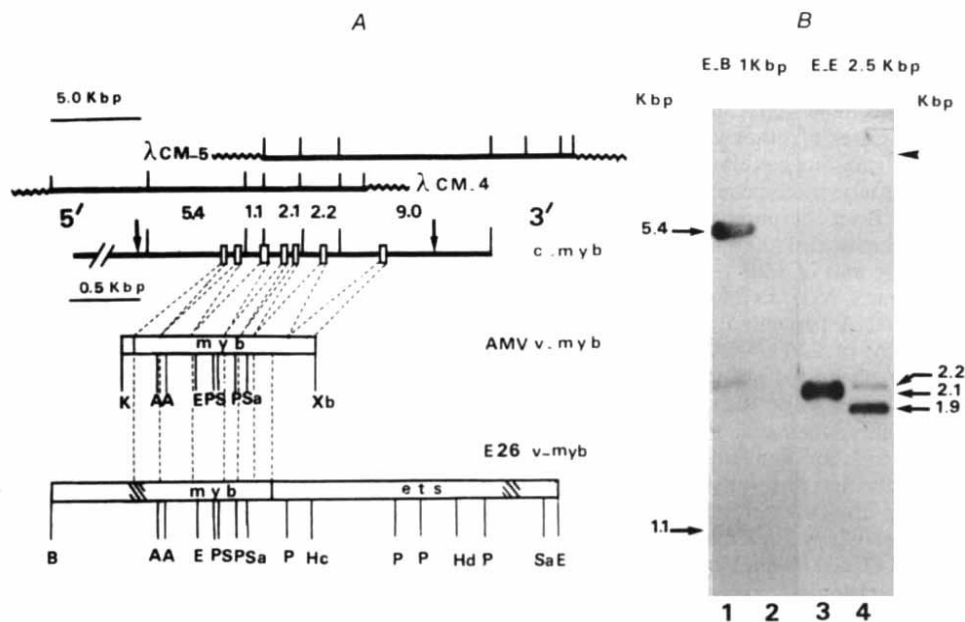


Fig. 2 *v-ets* has a cellular counterpart *c-ets*. The *v-ets* probe (probe a, see Fig. 1) was hybridized in non-stringent conditions to Southern blots of normal cellular DNAs restricted by *Eco*RI (lanes 2–4). DNAs used were from chick embryo fibroblasts (lanes 1, 2); Japanese quail fibroblasts (lane 3) and human placenta (lane 4). For normal chicken DNA, known to contain endogenous *env*-related sequences (as shown in lane 1 with a ³²P-labelled *env* probe), we checked that the *v-ets* probe, containing also some *env* sequences, yielded additional *ets*-specific bands (lane 2) not seen in lane 1.

Fig. 3 E26 *v-myb* is a truncated (at least in its 3' part) form of AMV *v-myb*.

A, Organization of the complete chicken *c-myb* locus and comparison of AMV *v-myb* and E26 *v-myb* restriction maps. From top to bottom: λ CM-4 and λ CM-5 are two phages¹⁵ isolated from a library of chicken DNA fragments inserted into the lambda phage Charon 4A. Vertical bars represent the *Eco*RI sites in the chicken DNA insert (solid line). Wavy lines, Charon 4A DNA. These two clones cover the complete *c-myb* locus, with the arrows defining the boundaries of the *c-myb* transcriptional unit¹⁴ and the open boxes the seven regions of homology (named E1 to E7 from 5' to 3')²¹ found between AMV *v-myb* and its cellular homologue. The restriction map of the two *v-myb* positive fragments in clone λ E26 Q1 (*Bam*HI-*Eco*RI 1 kb and *Eco*RI-*Eco*RI 2.5 kb; see Fig. 1) was determined with the following restriction endonucleases: *Ava*II (A) (first fragment) and *Hinc*II (Hc), *Hind*III (Hd), *Pst*I (P), *Sal*I (Sa) and *Sma*I (S) (second fragment). The 3' boundary between E26 *v-myb* and *v-ets* was precisely defined (|) by sequencing analyses of the *Sal*I-*Hinc*II 460 bp fragment (B. Debuire, unpublished results) and comparison with the unpublished AMV *v-myb* sequence^{7,20,21}. The 3' end (128 nucleotides) of E6 as well as all of E7 (120 nucleotides)²¹ were absent in E26 *v-myb*, and replaced by *ets* sequences in the same open reading frame (B. Debuire, manuscript in preparation). In contrast, the 5' boundary of E26 *v-myb* (with the *gag* gene) and the boundary between *v-ets* and *env* have been only roughly determined (⌘). **B**, Hybridization of E26 *v-myb* with *c-myb* recombinant phages. The two chicken *c-myb* recombinant phages (see panel A) λ CM-4 (lanes 1, 3) and λ CM-5 (lanes 2, 4) were digested with *Eco*RI, separated by agarose gel electrophoresis, transferred on to nitrocellulose and hybridized with the two ³²P-labelled E26 *v-myb* fragments. In case of the *Bam*HI-*Eco*RI 1-kbp probe, the blot was overexposed to visualize the 1.1-kbp fragment (this fragment is difficult to detect even with AMV *v-myb* probe since it contains only a 26-bp region of homology with *v-myb*²¹). The faint 2.2–2.1-kb band visualized in λ CM-4 (lane 1) is probably nonspecific because the equivalent fragments in λ CM-5 (2.2 and 1.9 kbp) did not hybridize (lane 2). The 2.5-kbp *Eco*RI probe detected the 2.2- and 2.1-kbp *c-myb* *Eco*RI fragment in λ CM-4 (lane 3). These fragments are better separated as 2.2- and 1.9-kb fragments in λ CM-5 (lane 4). The weak hybridization of the 2.2-kbp fragment is expected, as only half of E6 (140 nucleotides) is present in this probe. The arrowhead for lane 4 indicates the position of the 9-kbp *c-myb* *Eco*RI fragment containing the 3' distal 'exon' E7 not detected by the E26 *v-myb* probe.



v-ets also failed to hybridize in non-stringent conditions to murine *v-onc* genes *abl*¹⁸ and *ras*¹⁹ reported possibly to induce erythroid transformation (data not shown). We thus concluded that *c-ets* represented a distinct set of conserved nucleotide sequences.

It then became important to document whether the *ets* and *myb* sequences found contiguous in E26, represented two distinct cellular domains, or belonged to a single large locus in chicken DNA. Indeed, it was reported recently that the *c-myb* locus (contained within the arrows in Fig. 3A, third line) in the chicken genome was considerably larger than the truncated fragment of *myb* found in AMV^{14,20,21}. Thus, *v-ets* could be part of the *c-myb* locus, a larger portion of which would have been transduced by E26 than by AMV. This was not the case as the *EcoRI* restriction fragments of *c-ets* (Fig. 2, lane 2) differed in size from those contained within the *c-myb* locus (Fig. 3A, third line). Incidentally, the E26 *v-myb* domain appeared even smaller than in AMV (more truncated at least at the 3' end: see lines 4 and 5 in Fig. 3A, and Fig. 3B). We conclude that E26 contains two sets of cell-derived nucleotide sequences, one (E26 *v-myb*) derived from the *c-myb* locus, colinear with the AMV *v-myb* gene transduced by AMV but more truncated at least at its 3' end, and the other (*v-ets*) from a distinct cellular domain *c-ets* unrelated so far to any *c-onc* gene tested.

Finally, we showed that *c-ets* sequences were transcribed in chicken fibroblasts, in chicken bone marrow cells or MSB1 cells (a chicken line of T lymphoblasts transformed by Marek disease virus²²). In all cases we found a RNA species of 7.5 kb (as well as in some instances two faint 2.2- and 2.0-kb RNAs which are under investigation, Fig. 4, lanes 1–3). Indeed, analysis of the same RNA blots with a *v-myb* probe did not detect such bands but only the expected 4.0-kb *c-myb* mRNA species^{14,23}, as shown, for example, with MSB1 cells (Fig. 4, lane 4).

In conclusion, *c-ets* and *c-myb* represent two distinct loci in chicken cellular DNA, both transduced by a yet unknown mechanism into the E26 virus where they are translated from a genomic size viral RNA (no subgenomic RNA was demonstrated for E26, our unpublished results) as a triple hybrid (*gag-myb-ets*) protein of 135,000 molecular weight^{8,24,25}, a situation never seen before in acute transforming avian retroviruses. Although we cannot exclude the possibility that the complex oncogenic spectrum of E26 versus AMV could involve the *myb* gene alone (found truncated and perhaps mutated in E26), our experiments favour another alternative. We propose that the ability of E26 to cause erythroleukaemia in addition to myeloblastosis could result from the presence of a second oncogene, *v-ets*, within the viral genome. *v-ets* shares the properties found so far for other oncogenes¹⁰ (viral sequences translated into a protein, that are of cellular origin, conserved with evolution and are expressed as discrete mRNA species at some stages in normal cells). So far, all specific sequences with these properties found in retroviruses turned out to be oncogenes. That two distinct oncogenes are found co-existing in a same virus may not be coincidental as we recently described another retrovirus with two cell-derived specific sequences²⁶. A first such example, avian erythroblastosis virus (AEV) was shown to contain two *onc* genes (*erbA*, *erbB*) transcribed as two distinct mRNAs¹⁶ and translated into different proteins²⁷ (75,000 and 68,000 molecular weight, respectively), where *erbA* potentiates the transforming activity of *erbB* within the same erythroid lineage²⁸. In this respect in E26, *v-ets* could potentiate the transforming activity of E26 *v-myb*, as E26-transformed myeloblasts seem to be more tightly blocked in their differentiation than AMV-transformed myeloblasts⁴. In addition, the two specific genes described here for E26 may confer on this virus the property to transform cells already committed into both

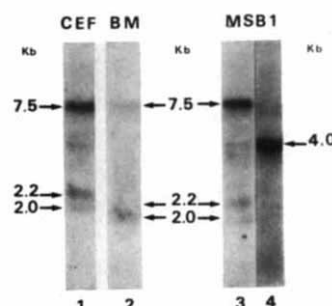


Fig. 4 Size of *c-ets* mRNA in chicken cells. Northern blots were prepared with polyadenylated mRNA and hybridized in stringent conditions (0.45 M NaCl, 41 °C, 50% formamide) with a *v-ets*-specific probe (the 720-bp *PstI* fragment, probe *b* in Fig. 1) (lanes 1–3). RNAs tested were: CEF, chicken embryo fibroblasts (lane 1); BM, bone-marrow (lane 2); MSB1, a cell line of T lymphoblasts transformed by Marek disease virus (MDV) (lane 3) that also expresses *c-myb* RNA at an elevated level^{22,23} as shown in lane 4 with a *myb* probe.

myeloid or erythroid lineages⁴ or uncommitted precursor cells still able to differentiate into these two lineages⁵. For further analyses along these lines, we are currently attempting site-specific mutagenesis in the E26 *myb* or *ets* sequences. Clearly, several experimental systems involving complementation of or synergy between more than one oncogene^{29–31} are emerging, which may lead to a better understanding of multi-step carcinogenesis.

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α -Thalassaemia caused by a polyadenylation signal mutation

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Most eukaryotic messenger RNAs have the sequence AAUAAA 11–30 nucleotides from the 3'-terminal poly(A) tract^{1,2}. Since this is the only significant sequence homology in the 3' non-coding region it has been suggested that it may be a recognition site for enzymes involved in polyadenylation and/or termination of polymerase II transcription^{2–4}. This idea is strengthened by observations on the effect of deletion mutations in or around the AATAAA sequence on polyadenylation of late simian virus 40 (SV40) mRNA; removal of this sequence prevents poly(A) addition³. Naturally occurring variants of this hexanucleotide are rare^{5–11} and hitherto their functional significance has not been assessed. We have now identified a human $\alpha 2$ -globin gene which contains a single point mutation in this hexanucleotide (AATAAA $\rightarrow$ AATAAG). The paired $\alpha 1$ gene on the same chromosome is completely inactivated by a frameshift mutation. This unique combination has enabled the expression of the mutant $\alpha 2$ gene to be studied *in vivo* where it has been found that the accumulated level of $\alpha 2$ -specific mRNA in erythroid cells is reduced. Furthermore, readthrough transcripts extending beyond the normal poly(A) addition site are detected in mRNA obtained from HeLa cells transfected with cloned DNA from the mutant $\alpha 2$ gene, suggesting that the single nucleotide change in the AATAAA sequence is the cause of its abnormal expression.

We have previously described a form of α -thalassaemia, found in Saudi Arabia, in which the combined output of the four α -globin genes in homozygotes is equivalent to one normal gene, suggesting that both α genes on each chromosome are expressed at a lower level than normal^{12,13}. Blot hybridization studies using a variety of restriction endonucleases and ³²P-labelled probes have shown that no detectable rearrangements occur over the entire α -globin gene complex¹³. To examine the $\alpha 1$ and $\alpha 2$ genes in greater detail, a recombinant containing both α genes was isolated from a library of size-fractionated *Bam*HI–*Eco*RI-cleaved DNA fragments cloned into the bacteriophage vector λ Charon 30 (refs 14, 15). Subsequently, 1.5-kilobase (kb) *Pst*I fragments containing the $\alpha 1$ and $\alpha 2$ genes were subcloned in pBR322 and fragments from these subclones were sequenced.

The defective $\alpha 1$ gene has a normal sequence apart from a single nucleotide deletion within the codon for amino acid 14 (Fig. 1). A deletion from triplet 14 (TGG), which normally codes for tryptophan, causes a frameshift mutation that also generates an in-phase termination codon (TGA) at the position of the 35th new codon; this mutation clearly inactivates the $\alpha 1$ -globin gene by causing premature termination of mRNA translation. The DNA sequence of the mutant $\alpha 2$ gene is identical to that of a normal $\alpha 2$ gene apart from a single nucleotide difference in the 3' non-coding region. This base change causes an A $\rightarrow$ G transition in the highly conserved polyadenylation signal sequence (AATAAA $\rightarrow$ AATAAG) (Fig. 1).

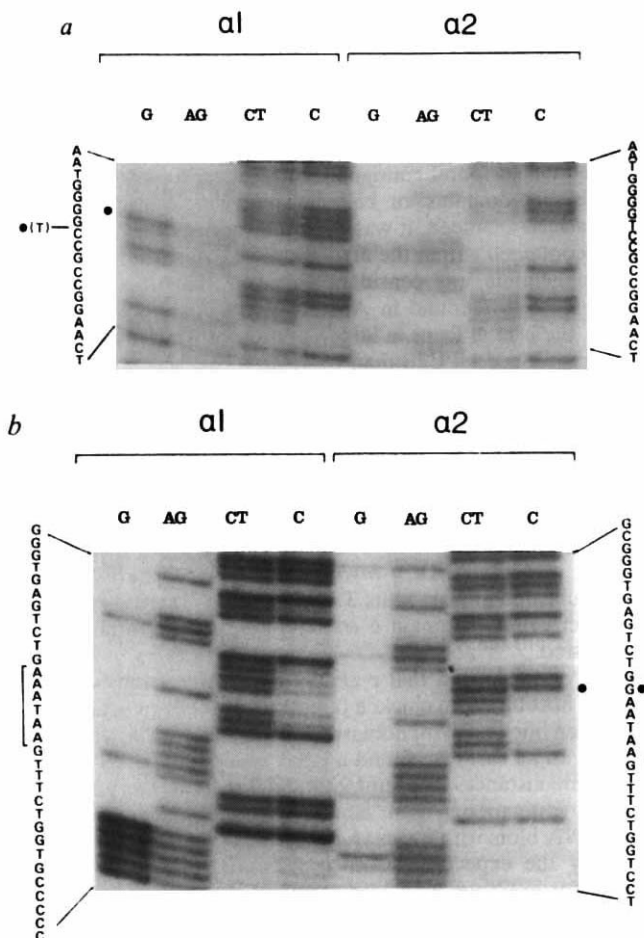


Fig. 1 DNA sequence of the mutant $\alpha 1$ (a) and $\alpha 2$ (b) globin genes. The sequences demonstrated are those of the complementary DNA strand with the sense strand written alongside; ● denotes variations from the normal sequences. Differences from the original published sequences^{31,32} were also noted in codons 54 and 123 of the $\alpha 2$ gene, positions 105, 110 and 112 in *IVS2* of the $\alpha 2$ gene and position 110 of *IVS2* of the $\alpha 1$ gene. These changes are consistent with the revised normal sequences for these genes^{33,34}. **Methods:** DNA fragments were labelled at their 3' termini and sequenced by the method of Maxam and Gilbert³⁵; the strategy was essentially that previously described for the normal $\alpha 1$ ³¹ and $\alpha 2$ ³² genes. Each gene was completely sequenced from –90 to +857 with respect to the mRNA cap site.

Analysis of cytoplasmic mRNA obtained from peripheral blood reticulocytes by cDNA:RNA soluble hybridization (refs 12, 13 and Fig. 2) showed that the accumulated level of α -specific RNA is reduced to about 15% of normal in homozygotes for this defect. To estimate the relative contributions of each α gene to this cytoplasmic RNA, $\alpha 1$ - and $\alpha 2$ -specific RNA transcripts were analysed by single-strand nuclease analysis. In normal adult reticulocytes $\alpha 2$ -specific globin RNA is more abundant than $\alpha 1$ ^{16,17} whereas in this case the opposite is true (Fig. 2). Estimates of RNA in this defect are consistent with accumulated levels of ~10% ($\alpha 1$) and 5% ($\alpha 2$) of the normal total mRNA. Since the $\alpha 1$ mRNA is non-functional, all of the α globin synthesized must be derived from the $\alpha 2$ genes.

The reduced level of $\alpha 1$ -specific RNA was expected by analogy with previously reported examples of frameshift and nonsense mutations in globin genes^{18–20}. To correlate the DNA sequence change in the poly(A) signal of the $\alpha 2$ -globin gene with the reduced level of $\alpha 2$ -specific mRNA, we studied the synthesis and processing of RNA from the mutant $\alpha 2$ gene. Because it was not possible to obtain bone marrow samples, and hence early erythroid precursors, we analysed the expression of the cloned $\alpha 2$ gene in HeLa cells, in which the pattern of *in vivo* transcription and processing can be mimicked^{21–24}. Four

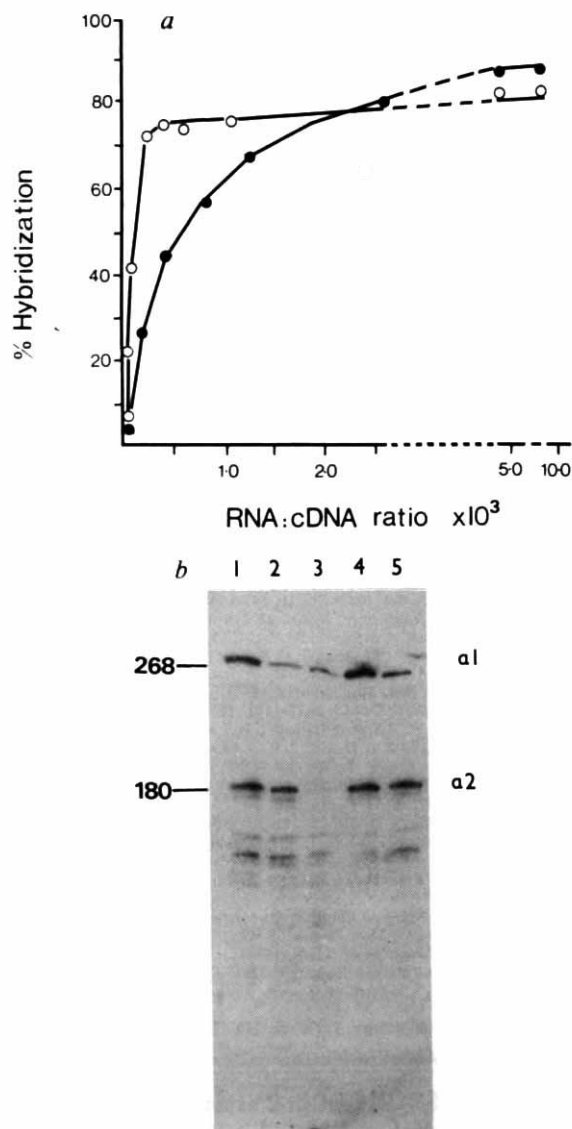


Fig. 2 Analysis of mRNA obtained from peripheral blood reticulocytes. *a*, Soluble hybridization studies (●, α; ○, β) in an individual homozygous for the Saudi Arabian α-thalassaemia defect. In this case the α/β globin mRNA ratio was 0.28 (compared with 2.0–2.2 in non-thalassaemic individuals). *b*, Single-strand nuclease analysis of the 3' ends of the α₁ (268 nucleotides) and α₂ (180 nucleotides) globin transcripts. Lane 2, non-thalassaemic individual; lanes 1, 5, two individuals with uncharacterized forms of non-deletion α-thalassaemia; lane 3, an individual with α-thalassaemia in which only the α₁ gene is fully functional; lane 4, a homozygote for the Saudi Arabian α-thalassaemia defect. The minor bands at approximately 160 nucleotides are due to double-stranded activity of the nuclease and correspond to an A + T-rich region in the hybrid (5'-AAATACCGTTAA-3').

Methods: The relative amounts of α- and β-globin mRNA were determined as previously described³⁶ using ³H-labelled cDNA α (●) and cDNA β (○). Analysis of the α₁ and α₂ mRNA transcripts was as described by Orkin and Goff¹⁶. 200 units of mung bean nuclease (P-L Laboratories) were used per sample (5 μg total RNA) rather than S₁ nuclease as originally described.

α-globin/SV40 plasmids containing normal and mutant α genes were examined (Fig. 3). Analysis of the 3' end of the RNA transcripts isolated from these cells indicated that approximately equivalent amounts of normal and mutant RNAs are produced in this transient expression system. However, whereas the 3' extent of the normal α₂ mRNA is marked by the usual poly(A) addition site, the majority of the α mRNA in both nuclear and cytoplasmic preparations from HeLa cells transfected with the mutant α₂ gene includes sequences beyond this point. An S₁

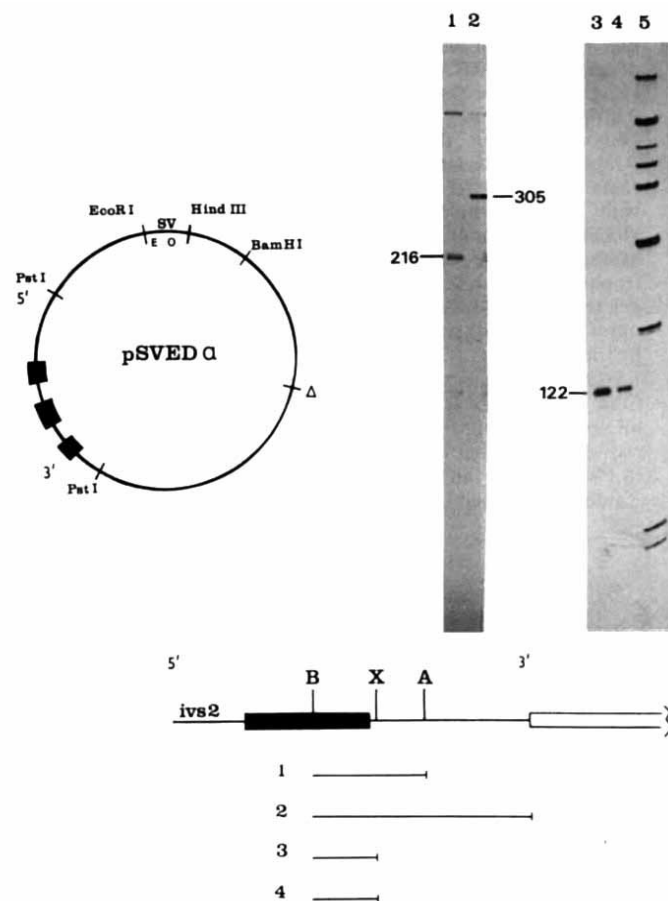


Fig. 3 Expression of the α₁- and α₂-globin genes in HeLa cells. The cloning vector (pSVED) is based on pBR322 but lacks nucleotides 1,420–2,490 (Δ) and contains an SV40 DNA fragment inserted between the EcoRI and HindIII sites (SV). This fragment includes both the SV40 origin of replication (O) and enhancer sequences (E). PstI fragments of normal (W) and mutant (M) α₁ and α₂ genes were subcloned into this vector (pSVED α₁ W, pSVED α₁ M, pSVED α₂ W, pSVED α₂ M). The orientation of the α genes is away from the SV40 origin insert so that the α-gene promoter is close to the SV40 enhancer sequence, and reading off the other DNA strand to the SV40 early promoter. Total RNA from: lane 1, pSVED α₂ W; lane 2, pSVED α₂ M; lane 3, pSVED α₁ W; lane 4, pSVED α₁ M; lane 5, HinflI-cut pBR322 markers. Identical extended transcripts from pSVED α₂ M were seen in total, nuclear and cytoplasmic RNA preparations (data not shown). In the diagram below, ■ indicates the third exon of the α-globin gene and □ indicates vector sequence. B indicates BstEII and X the mismatch between α₁ and α₂ sequences 15 nucleotides beyond the TAA stop codon³¹. A denotes the poly(A) addition site in the 3'-flanking region of the α genes.

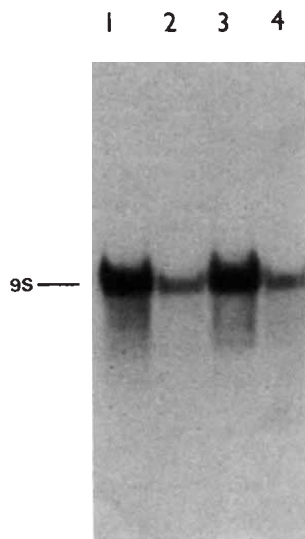
Methods: The four pSVEDα plasmids were introduced independently into HeLa cells by calcium phosphate precipitation. Total cellular, or separate nuclear and cytoplasmic RNAs were isolated 3 days after transfection²⁴. To perform S₁ mapping on these RNAs, a 600-base pair BstEII fragment, including the 3' end of the α₂ gene and extending into the 3'-flanking sequence, was end-labelled with [α-³²P]dGTP, strand separated and the anti-mRNA-sense strand was used as a probe. S₁ analyses were performed as described in ref. 37 using equivalent amounts of RNA from each experiment. The products were run on 6%, 7M urea polyacrylamide gels.

nuclease-protected fragment corresponding to a mismatch position at which the 3' end of the mutant α₂ globin gene is joined to the vector DNA shows that α₂ transcripts extend through the entire 3'-flanking sequence of the mutant gene included in the PstI insert of pSVEDα₂M (Fig. 3) and presumably terminate in the vector DNA sequence.

The precise mechanism of termination of transcription and polyadenylation in eukaryotes is not known. However, it has been shown in several different systems that polymerase II

Fig. 4 Northern blot analysis of glyoxylated RNA samples (5 µg) from non-thalassaemic individuals (lanes 1, 3), a homozygote for the Saudi Arabian α -thalassaemia defect (lane 2) and another individual with only one functional α -globin gene (lane 4).

Methods: RNA was electrophoresed in 1.5% agarose gel, transferred to diazobenzyl-oxyethyl (DBM) paper³⁸ and hybridized to a ³²P-labelled 1.5-kb *Pst*I fragment prepared from the genomic α subclone pRB1³⁹. The DBM paper was washed in stringent conditions (0.1×SSC at 68°C) and autoradiographed for 24 h.



transcription proceeds past the site for poly(A) addition²⁵⁻²⁹ and it has therefore been suggested that two steps are involved—first an endonucleolytic cleavage of the primary mRNA transcript and then the addition of a poly(A) tail^{2-4,25-30}. The high degree of conservation of the AATAAA sequence at the 3' end of eukaryotic mRNAs suggests that it is an important component of the recognition process. Exceptions to the AATAAA sequence have been documented [ATATAA (refs 5-8), AGTAAA (refs 9, 10), TATAAG (ref. 11)] although their relative efficiency as recognition sites has not been assessed. The data presented here indicate that a point mutation in the AATAAA sequence interferes with the normal termination/polyadenylation process in HeLa cells, probably by a failure to cleave transcribed RNA efficiently. Similar extended transcripts have also been demonstrated in RNA obtained from HeLa cells transfected with the human $\psi\alpha$ gene (AATGAA) (E. Whitelaw and N. J. Proudfoot, in preparation), suggesting that single point mutations within this region are sufficient to alter its function radically even though this hexanucleotide may not be the entire recognition sequence^{2,4}.

Northern blot analysis of α -globin mRNA obtained from the peripheral blood reticulocytes of a homozygote shows that it is identical in size to that from normal reticulocytes (Fig. 4). Furthermore, S₁-nuclease analysis, using a probe extending into the 3'-flanking region of the $\alpha 2$ gene, failed to demonstrate extended transcripts in this RNA (data not shown). It is possible that abnormal transcripts are not transported to the cytoplasm in erythroid precursors, although this was not the case in HeLa cells. Alternatively, the extended transcripts may be less stable than normally processed mRNA. This would be consistent with the fact that abnormal transcripts were not observed *in vivo*; possibly because they are relatively unstable they are only present in very small amounts in late erythroid cells. The mRNA that we have detected in reticulocytes therefore represents those transcripts which have been cleaved correctly at the poly(A) addition site. Since these processed transcripts were sufficiently stable to be detected in reticulocytes it seems most likely that they are polyadenylated in the normal way.

Analysis of specific mutations in highly conserved regions of eukaryotic genes has provided a better understanding of the relationship between DNA sequence and function. Hitherto, mutations involving initiation of transcription, RNA splicing and mRNA translation have been reported. The characterization of the naturally occurring poly(A) signal mutation described here provides further support for the significance of this highly conserved sequence in the normal expression of eukaryotic genes.

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Nucleotide sequence evidence for rapid genotypic shifts in the bovine mitochondrial DNA D-loop

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Mitochondrial DNA (mtDNA) is unusual in its rapid rate of evolution¹ and high level of intraspecies sequence variation². The latter is thought to be related to the strict maternal inheritance of mtDNA, which effectively isolates within a species mitochondrial gene pools that accumulate mutations and vary independently³. A fundamental and as yet unexplained aspect of this process is how, in the face of somatic and germ-line mtDNA ploidy of 10³ to 10⁵ (refs 4, 5), individual variant mtDNA molecules resulting from mutational events can come to dominate the large intracellular mtDNA population so rapidly. To help answer this question, we have determined here the nucleotide sequence of all or part of the D-loop region in 14 maternally related Holstein cows. Four different D-loop sequences can be distinguished in the mtDNA of these animals. One explanation is that multiple mitochondrial genotypes existed in the maternal germ line and that expansion or segregation of one of these genotypes during oogenesis or early development led to the rapid genotypic shifts observed.

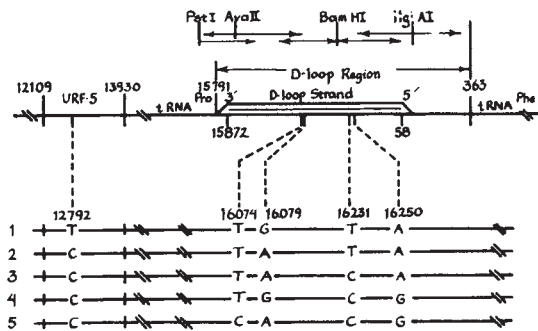


Fig. 1 Positions of the variable URF-5 and D-loop region (L-strand) nucleotides in the bovine mtDNA genotypes. Lane 1, cows H496, H512, H576, H634, H709, H737B, H919B, H997B and H1009B (see Fig. 2 for lineage); lane 2, H455; lane 3, the bovine mtDNA sequence of Anderson *et al.*⁷; lane 4, H949B; lane 5, H393, H493 and H624. Solid lines indicate nucleotide sequences identical to that of Anderson *et al.*⁷ The bovine mitochondrial genome is 16,338 bp in length. Nucleotide positions are shown for the URF-5 gene, the variable URF-5 nucleotide, the extent of the D-loop region, the extent of the longest D-loop strand and its polarity (P.D.O. and W.W.H., unpublished data), and the variable D-loop nucleotides. At the top is shown the sequencing strategy for the D-loop. The double-headed arrow from the *Bam*HI site indicates that the sequence of both strands was determined for some animals.

Methods: MtDNA from the livers or brains of culled animals or from liver biopsy specimens was prepared from purified mitochondria as described previously¹⁸. *Pst*I digestion of bovine mtDNA yields two fragments, *Bam*HI and *Eco*RI digestions yield three fragments each¹⁸. These were cloned into the *Pst*I and *Bam*HI sites, respectively, of pBR322 and into the *Eco*RI site of pACYC184 by standard techniques^{19,20}. DNA was isolated from two L cultures by a modified alkaline SDS lysis procedure²¹ followed by acridine yellow-agarose column chromatography²². The DNA sequence was determined by the chemical technique of Maxam and Gilbert²³. Routinely, sequences were determined from a ³²P-labelled 3' end. End-labelling was accomplished using [α -³²P]dGTP and *Escherichia coli* DNA polymerase I large fragment²⁴. (Some sequences from the *Bam*HI site were also determined from ³²P-labelled 5' ends using [γ -³²P]ATP and polynucleotide kinase. To eliminate the possibility of cloning artefacts, sequences around the *Bam*HI site were determined from several independently isolated clones of several animals: H634 (six clones), H493 (four clones), H1009B (three clones), and H949B (two clones).

For any animal, all clones gave an identical sequence.

We recently reported an example of mtDNA sequence variation within a single maternal lineage of Holstein cows⁶ which is characterized by a single base transition in coding region URF-5. This suggested that at least limited variation can occur among maternally related animals. It was unclear, however, whether there were just the two mitochondrial genotypes or many genotypes whose nucleotide sequences at URF-5 fell into two classes. To determine more accurately the number of mitochondrial genotypes, it is necessary to determine if any other nucleotide sequence differences are associated with each of the URF-5 variants. If so, the numbers of different sets of co-inheriting sequences would be a more accurate reflection of the true mitochondrial heterogeneity within this maternal lineage. Because the D-loop region (a 910 bp sequence located between the tRNA^{Pro} and tRNA^{Phe} genes, ~3,000 base pairs (bp) away from URF-5⁷) is reported to be the least conserved region of the mtDNA molecule⁷⁻⁹, it is a favourable region to search for such sequence changes. The nucleotide sequence of all but the 5'-terminal 12 nucleotides of this region was determined from cloned mtDNA of seven animals. In an additional seven animals the 400-bp region surrounding the D-loop *Bam*HI site was sequenced.

The sequenced regions fall into four classes, distinguished by variation at four nucleotide positions (Fig. 1). These data, combined with our previous data for URF-5 variation, can be

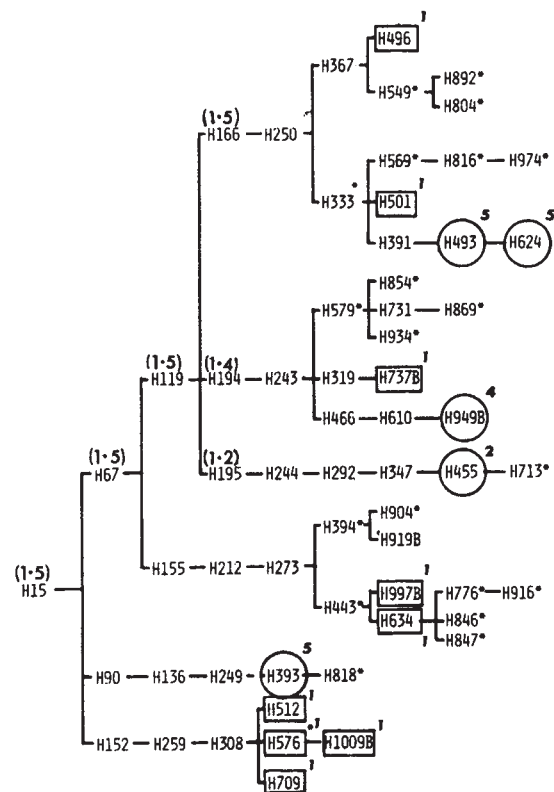


Fig. 2 Holstein cow maternal lineage from which tissue for the present study was taken. Circles indicate animals of the 12792-C genotype; boxes indicate animals of the 12792-T genotype; asterisks indicate animals presently alive in the herd; suffix 'B' indicates bull calves. Numbers above the boxed or circled animal numbers indicate the observed mitochondrial genotype as abbreviated in Fig. 1. Similar numbers in brackets above some other animal numbers are the predicted mitochondrial genotypes (see text).

Methods: With the exception of the founding maternal ancestor, Holstein H15, all animals in the H15 maternal lineage have been born, bred and maintained at the University of Florida Dairy Research Unit. Three sets of records exist on the birth and genetics of every animal in the herd and these records for all animals in the H15 lineage are consistent with the data shown. Calf-switching between dams before animal tagging could theoretically lead to an artificial mixing of genotypes within a lineage. Three types of data refute this possibility. First, 12792-T genotype animals are present only in the H15 lineage. If calf-switching were common, this genotype would be present in other maternal lineages in the herd. Second, we have similarly analysed another Holstein lineage of 51 animals whose mtDNA exhibits a restriction enzyme digestion pattern distinguishable from patterns of all other available Holstein lineages¹³. In this lineage we find no evidence for mitochondrial genotype mixing as would be expected if calf switching were as common as the H15 lineage data would require. Third, in dairy herds maintained for breeding studies, calf switching is extremely rare (C. J. Wilcox, personal communication) both because of close management and because dams are extremely reluctant to switch. Thus, consideration of record-keeping procedures, birth records, mitochondrial genotypes between maternal lineages, and Holstein cow maternal behaviour all attest to the accuracy of the H15 lineage.

summarized as follows: (1) Clones of the nine animals whose mtDNAs contain a thymine residue at position 12,792 in URF-5 (12792-T genotype) have an identical D-loop sequence (genotype 1, Fig. 1). (2) Clones of the five animals whose mtDNA has a cytosine residue at position 12,792 in URF-5 (12792-C genotype) exhibit three different types of D-loop sequences characterized by various combinations of base transitions at positions 16,074, 16,079, 16,231 and 16,250 (genotypes 2, 4 and 5, Fig. 1).

Because the number of genotypes is a significant fraction of the number of animals examined, it seems probable that more genotypes exist within the lineage, particularly in 12792-C animals. Another bovine mtDNA D-loop sequence has been reported by Anderson *et al.*⁷ (genotype 3, Fig. 1). That D-loop sequence differs by single base transitions at sites 16,231 and 16,079 from genotypes 1 and 4 respectively.

This D-loop sequence variation within a maternal lineage could, in theory, arise from several sources. The base transitions might reflect some novel mutational mechanism characteristic of the D-loop, which results in rapid sequence variation. Alternatively, nuclear genes may predispose some species, breeds or lineages to mtDNA variation. However, the most appealing explanation arises from the polyploid nature of mtDNA itself and the structure of its D-loop. The observation of four mitochondrial genotypes in one maternal lineage strongly suggests that some combination of the four genotypes co-existed in the common maternal ancestor H15 or a maternal relation and that the genotypes have subsequently segregated into their progeny. This implies that oocytes and/or somatic tissues of these animals contained a heterogeneous population of mtDNA molecules. A recent independent report¹⁰ describes the isolation of two clones of human placental mtDNA containing D-loop sequences from the same individual which differ by one *Hae*III site, suggesting that a heteroplasmic state may also exist in human tissue.

Assuming that animals may be heteroplasmic, a possible mechanism for the origin of D-loop sequence variation can be suggested on the basis of three facts. First, all four base substitutions occur within the triple-stranded region of the D-loop (between positions 15,872 and 58) (P.D.O. and W.W.H., unpublished observations). Second, from previous interspecies comparisons⁷⁻⁹ only one of the variable bases (position 16,079) is within an evolutionarily conserved block and it is therefore likely that three, if not all four, base changes are functionally neutral. Third, it is known that during replication, the 7S DNA which creates the D-loop is resynthesized and displaced many more times than it is elongated into daughter strands¹¹. Therefore, in cells that are heteroplasmic for at least two D-loop genotypes, a displaced parental strand or 7S DNA strand of one genotype could invade the D-loop of a sister genotype and create as many as four single base mismatches. If such mismatches can be randomly repaired, all base substitution genotypes intermediate between the parental genotypes could be formed. Amplification and segregation in subsequent generations may then lead to the appearance of new D-loop genotypes in the progeny. Apparently, this gene conversion event can take place over short distances, because nucleotides as few as five bases apart appear to be separable (for example, positions 16,074 and 16,079). This result is different from the type of long patch repair thought to give rise to asymmetric gene conversion in fungi and prokaryotes¹² and may reflect the limited DNA repair capacity of mammalian mitochondria¹³. This D-loop gene conversion mechanism is consistent with the observation that changes apparently occur only within the triple-stranded region of the D-loop, only the same four bases change, and only base transitions are observed.

The above considerations allow an assignment of probable mitochondrial genotypes to pivotal animals within the H15 lineage (Fig. 2). Because all early branches of the lineage defined

by H67, H90 and H152 contain individual animals with either genotypes 1 or 5 (Fig. 1), H15 is predicted to have been heteroplasmic for these two genotypes. Assuming H15 had an approximately equal proportion of genotypes 1 and 5 (reflecting the observed proportions of genotypes 1 and 5) the number of sexual generations (20 to 50 germ-line cell generations per sexual generation) required to produce the observed relatively pure mitochondrial genotypes from a heteroplasmic ancestor implicit in our analysis is within the limits of a recently developed random segregation model for cytoplasmic genes (ref. 14 and C. W. Birky, personal communication). Utilizing the gene conversion hypothesis, the D-loop sequences of H949B (genotype 4) and H455 (genotype 2) may have then arisen within a 1-5 heteroplasmic ancestor, followed by segregation in its progeny. We note that H194 and H195 are non-identical twins. It is therefore possible that both genotypes 2 and 4 arose in H119 and that subsequent mitochondrial segregation in her germ-line cells led to the establishment of genotypes 2 and 4 in H194 and H195 respectively.

A final question as to the generality of such rapid shifts in mitochondrial genotypes bears consideration. We have surveyed eight other Holstein and five Jersey cow lineages by restriction enzyme digestion (ref. 15 and our unpublished observations). One of these lineages, a Jersey line, also shows a restriction site polymorphism. More nucleotide sequence analysis needs to be done, particularly in the D-loop region, before this lineage can be said to exhibit the type of shifts seen in H15. However, this does suggest that mtDNA variation between maternally related animals may not be as rare as might be inferred from previous work (for example, ref. 3), particularly if D-loop nucleotide sequences are examined. This might be consistent with the recent analysis of D-loop sequences^{10,16} and length polymorphism¹⁷ in human mtDNA indicating a preference for D-loop variation.

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Science transcended

George M. Marsden

The Origins of Life: Evolution as Creation.

By Hoimar v. Ditfurth. Translated by Peter Heinegg.

Harper & Row: 1983. Pp.280. £9.50, \$14.95.

FOR well over a century a popular belief has been that religion and science stand at opposite ends in the cultural evolution of human intelligence. Gradually but irreversibly, according to this view, primitive religious superstitions are replaced by empirically based scientific conclusions. Although most scholars have abandoned the positivistic aspects, the concept of an essential conflict between science and religion has been so indelibly imprinted on the collective intelligence of modern people that it will be difficult to displace.

Hoimar v. Ditfurth, well known in Germany as an author of popular scientific books, attempts to destroy this mythology with the weapons of science itself. In sometimes brilliant and fascinating discussion, Ditfurth argues that today's science is highly conducive to religious belief and provides strong presumptions for faith in a "beyond" or a transcendent creative intelligence.

At his most effective, Ditfurth meets objections to the belief in a transcendent mind that guides the universe. Evolutionary theory of the development of mind together with our knowledge that the universe could well include billions of intelligent life forms, suggests that our own understanding of reality is far from definitive. The mysteries on the edges of our scientific inquiry suggest that there are other dimensions to which our consciousness does not yet have direct access.

This concept can be supported by an evolutionary epistemology in which the categories for thought that Kant believed were *a priori* postulates, are considered to have developed *a posteriori* in the collective consciousness of the species. Ditfurth uses the analogy of chickens which have imprinted in their pristine intelligence fears of their mortal enemies. So, he argues, we humans have evolved inborn mental structures that compel us to perceive reality through categories such as spatiality, temporality or causality. Our conclusions based on this "inborn" intelligence seem so compelling because they have been well attuned to ordinary circumstances. Nonetheless, twentieth-century science and philosophy of science both show how arbitrary and hypothetical is even the most sophisticated of this empirical intelligence. We have a presumption, then, for supposing there are dimensions of reality to which our minds are not fully attuned.

There is another presumption, based on the mystery of a universe structured to

produce life. How is it, Ditfurth asks, that when the universe emerged from the big bang it contained the capability of producing new forms, including life forms? This astounding capability, he argues, makes sense best if we view ongoing evolution as part of creation. That a transcendent mind is guiding the natural processes he finds confirmed by the fact that evolution creates more and more intelligence and consciousness. The cosmos is thus moving in the direction of blending with the principle of mind.

So Ditfurth has produced a natural theology, a rare enterprise in this century. His outlook is deistic in positing a creator as necessary to account for the incredible design of nature, but not deistic in that the transcendent maintains an immanent relationship with the universe. Moreover, in contrast to the deist's difficulties in explaining why God, "the perfect watchmaker", should have permitted imperfections, Ditfurth suggests a way of resolving the problem of evil. The imperfections of creation are necessary to the evolutionary creative process. While chance developments may bring much pain of constant readjustments, they also provide the freedom that keeps the processes going. An evolutionary outlook, Ditfurth emphasizes, balances chance and lawfulness. Pure chance would lead to meaningless chaos. Total governance by fixed laws would lead to equally meaningless automatism and stagnation. In combination, however, the laws of nature govern the course of chance.

Evolutionary theory provides one other presumption favouring religious belief. The collective intelligence accumulated by species is typically far superior to that based on the experience of any of its individual members. The same may apply to aspects of human intelligence. Some social theorists, for instance, have suggested the politically comforting theory that cultural systems may survive because they embody a supra-intelligence superior to that of any of the individuals who govern. So it may be dangerous to overthrow long-established cultural practices and taboos just because we can not understand the reasons for them. Similarly, we might presume that some long-standing religious beliefs may be based on insight superior to those grounded on mere empirical judgement. So Ditfurth neatly reverses the usual evolutionary assumptions about science and religion. Rather than being a vestige of primitivism, religious beliefs may embody

superior insights not yet fully available to our conscious intelligence.

Unfortunately, although Ditfurth has some highly suggestive insights on religion based on evolutionary theory, he considerably overestimates their originality. The first section of his book is written with a particularly arrogant tone, as though he were the first person in the twentieth century to realize that science and religion can readily be harmonized. He ignores the many modern theologians who have viewed scientific and religious beliefs as complementary, not contradictory.

Moreover, despite some genuine originality, Ditfurth overestimates the novelty of his efforts to integrate specific evolutionary insights and theology. For the past century a host of theologians and philosophers have been proposing variations on this theme. Ditfurth clearly has an advantage of a firm command of scientific literature. But he tends to present his work as absolute insights concerning the evolutionary processes. In the last analysis, God for him is evolution.

So, despite his claims that readers will not be "asked to doubt even a fraction of their religious faith", Ditfurth dismisses out of hand all traditional theologies in favour of his evolutionary formulations. Traditional theologies, he assumes, are hopelessly outdated and their perpetuation is the cause of the decline of the churches. Only a core belief in a transcendent creator, he argues, is a superior primal insight that is consistent with current scientific thought.

But does not this confidence in interpreting current evolutionary theory conflict with Ditfurth's own warnings not to overestimate the present state of human intelligence? Certainly, as far as Christianity is concerned, the decline of churches today is not obviously attributable to the promotion of outmoded theologies. Rather, the churches that have declined most dramatically are typically those that have made the greatest efforts to keep their theologies up-to-date. Meanwhile the churches that grow are usually those with more-or-less traditional theologies. Of course, popularity is no evidence of correctness of belief. But Ditfurth's insights might support more traditional beliefs than he is ready to acknowledge. Evolution after all is largely a conservative process, preserving accumulated achievements while adjusting them to current challenges. So might not a more consistent evolutionary approach to religion be to conserve the vast majority of past developments while adjusting them to new conditions and insights? Evolutionary theory by itself can not finally adjudicate on such issues. □

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Chandrasekhar on black holes

Roger Penrose

The Mathematical Theory of Black Holes.

By S. Chandrasekhar.

Oxford University Press: 1983. Pp.646.
£55, \$110.

SINCE the discovery of quasars roughly twenty years ago, black holes have become increasingly accepted as real physical objects. Theoretical discussion of them has shifted from general arguments (e.g. singularity theorems), aimed at proving their existence from very broad assumptions, to detailed consideration of their structure.

The very possibility of such detailed studies stems from some remarkable mathematical results concerning black-hole solutions of Einstein's equations. In 1963, Roy Kerr found the relevant solutions of Einstein's equations. In 1968, Israel's result concerning the uniqueness of Schwarzschild's solution led some people to conjecture that Kerr's solutions actually represent the most general possible end-points of gravitational collapse to a black hole. In the years 1972-1975, primarily through the work of Carter, Hawking and Robinson, this conjecture was established

in its essentials. We have thus been presented with an astonishing situation, perhaps almost unique in science, where a virtually complete theoretical description exists for a class of physical objects (black holes), while definitive observations are, as yet, almost non-existent.

It is fitting, therefore, that Chandrasekhar's new book *The Mathematical Theory of Black Holes* should concern itself, to a very considerable extent, with the geometry of Kerr's solutions and the many astonishing mathematical properties they exhibit. There is no doubt in my mind that this book is a masterpiece. Chandrasekhar has become the established world expert in the sort of material it is largely concerned with: perturbation analysis, separability of the equations, scattering of incident waves, etc. The equations are often extremely complicated, but the book is, nevertheless, extremely readable. The author's enthusiasm and wonder at the many

remarkable properties his equations possess is infectious, and serves to carry us through the more difficult passages. He does not hesitate to wax poetic where appropriate; "... properties which have endowed the Kerr metric with an aura of the miraculous"; or "... the analysis has led us into a realm of the rococo: splendid, joyful, and immensely ornate".

The book makes no attempt to cover all aspects of black holes. Observational matters, accretion disc theory, etc. are not considered, nor are the more esoteric mathematical techniques needed for, e.g., singularity theorems. But in all other respects it is remarkably complete and thorough. There are numerous tables of numerical data, graphs and diagrams, as well as equations. It is beautifully written and well-presented — and clearly intended to last a long time. It will. □

Roger Penrose is Rouse Ball Professor of Mathematics at the University of Oxford.

Second light

Peter Knight

The Quantum Theory of Light, 2nd Edn.

By Rodney Loudon.

Oxford University Press: 1983. Pp.393.
Hbk £30, \$59.95; pbk £15, \$24.95.

It is a pleasure to welcome a new edition of Rodney Loudon's standard text on the quantum theory of light. In the ten years since the first edition appeared, this book has become essential reading for anyone working in the new and dynamic field of quantum optics. A whole generation of students has been brought up on its clear and down-to-earth approach. The early chapters of the book can be read with profit by final year undergraduates interested in modern optics with only a modest prior undergraduate knowledge. Later chapters present a unified view of the quantum theory of coherence, photon counting, light scattering, laser action and nonlinear optics suitable for students beginning research. Some experimental details intrude where necessary although it remains a pedagogic text on the basic theory of photon physics. An experimentalist would need to supplement his reading with a more device-orientated text.

In the past decade, the great changes in the field have been stimulated by the development of narrow bandwidth tunable lasers. The dynamical evolution of atoms strongly and coherently coupled to radiation fields was hardly touched in the first edition. The new edition contains a careful discussion of Rabi population oscillations, Optical Bloch equations, strong-field resonance fluorescence, photon antibunching and much else. A large number of new figures and references appear in this edition. Problems and exer-

cises are scattered throughout the text. The discussion of quantum damping theory in the first edition has been much improved by the inclusion of the Heisenberg picture theory of radiative interactions as developed in Rochester and elsewhere.

I would have liked the author to elaborate a little on the usefulness of coherent states of the radiation field in dynamical problems in quantum optics, particularly in understanding the semiclassical limit. The diagonal coherent state representation, Fokker-Planck equations and master equations are not included, perhaps in keeping with the author's desire to use the simplest reliable techniques. The effects of laser bandwidths on resonant excitation are also ignored, which I think is unfortunate as I am sure the complicated subject of multiplicative stochastic processes in nonlinear optics could have been presented here with the author's usual flair. Perhaps he could consider these for the third edition? The sections on photon statistics in nonlinear optics, a field to which Professor Loudon has made notable contributions, has been largely rewritten and expanded. A discussion of the nonclassical aspects of light and of the photon description of interference are welcome additions, and demonstrate how hard it is to observe the true quantum nature of photons.

With a paperback edition of this well-printed book available at a reasonable price, I expect the second edition to be as popular with students as the first. New research students are lucky to have such a clear guide to their field when compared with the deadly quantum field theory texts of twenty years ago, with which my generation had to cope. □

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Sexual selection and plants

Deborah Charlesworth

Mate Choice in Plants: Tactics, Mechanisms and Consequences.

By Mary F. Willson and Nancy Burley.
Princeton University Press: 1983. Pp.251.
Hbk \$45.50, £30.30; pbk \$16.25, £10.90.

THIS book is not really about mate choice, but about the interesting and neglected problem of sexual selection in plants. Indeed, the sections on mate choice are in some respects the weakest part of the book, which is essentially a series of speculations that certain features of plant reproductive biology may have evolved due to sexual selection. Competition between individuals functioning as males for successful fertilization of ovules is discussed in various contexts, and the authors suggest that this may have played an important role in the evolution of delays in fertilization, cleavage polyembryony in gymnosperms, and double fertilization in angiosperms, as well as having the more obvious effect of determining pollen quantity and characteristics such as size and nutrient content. It is a pity that pollen competition is not well reviewed. Pollen production is mentioned on p.45, where it is wrongly stated that "a two-fold increase of pollen released by an individual results in only a 50% increase in the number of stigmas pollinated". Competition during pollination is mentioned extremely briefly; on p.55, four lines cover the evidence for competition between pollen grains of different genetic constitutions, with no reference to the classic *Oenothera* data.

Discrimination between genetically different pollen, during the female functions of reproduction, is assumed to exist, presumably on the grounds that there must be some evolutionary response to processes that have evolved as a result of male competition. But the case for differential abortion of zygotes based on any factor other than the degree of inbreeding of the

progeny, is very weak. The statement (p.50) that "if outbreeding were of selective advantage per se, the fitness gains for male and female should be the same", is typical of the unclear arguments in this book, and is not even correct except in a totally outbreeding species. The statement (p.47) that "if selfing were strongly disadvantageous, we might expect higher levels of self-sterility than seem to be the case" is also unconvincing without further explanation. Perhaps this view that avoidance of inbreeding is not important in plants explains why self-incompatibility is scarcely mentioned, except indirectly as "pre-zygotic recognition of pollen types". There is no mention at all of the possible existence of self-incompatibility acting after fertilization. The discussion of simple polyembryony in gymnosperms would seem much more enlightening if viewed as a mechanism for preventing loss of an ovule (and the resources invested in it), due to inviability of the zygote it gives rise to, which would be most likely to occur because of inbreeding.

The weakness of the dismissal of inbreeding avoidance would be less serious if the book presented good evidence for other factors influencing chance of abortion of zygotes, but the authors seem simply to assume that these exist. They refer to "genetic complementarity", but do not explain what this is; apparently it can result in differences in survival of progeny of reciprocal crosses. Almost 10% of the book is devoted to a list of phenomena related to fertilization (in animals and plants) that could be the basis for differential chances of success of the fertilization products, but there is no case where female discrimination is known to occur. For these reasons, the parts of this book dealing with mate choice are unsatisfactory. There may well be no true opportunity for mate choice in plants, apart from the well known outbreeding systems such as self-incompatibility, but merely a range of adaptations by which plants have responded to this lack. □

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All about antlers

Malcolm Maden

Deer Antlers: Regeneration, Function and Evolution.

By Richard J. Goss.
Academic: 1983. Pp.316. \$45, £32.

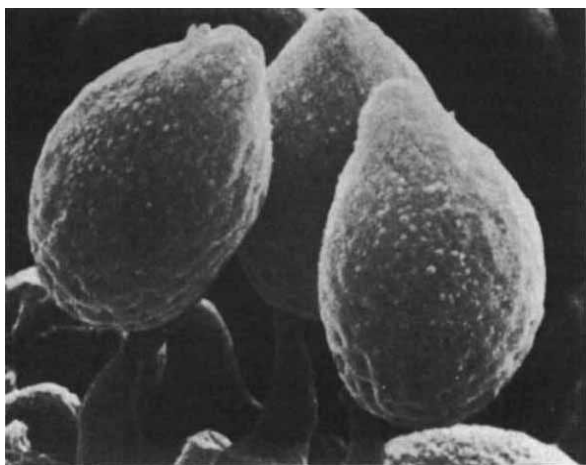
IF you want to know how the Lapps castrate reindeer, which is the only deer to possess a gall bladder, which deer is the national animal of Chile, which species only has two mammary glands, which is the world's handsomest deer, the deer with the lowest chromosome number of any mammal etc., then this book will answer your questions. In this way the author solves the problem he poses in the preface: how so narrow a subject as deer antlers could fill an entire volume, not to mention interest very many readers.

Goss has had a self-confessed 25-year love affair with deer and his book begins with a description of the characteristics of every species of deer and their global distribution. The following section on the evolution of deer and their antlers kept me amused thanks to the usual ingenuity of evolutionary biologists. Perhaps the most bizarre theory for antler evolution is that they evolved to function as thermal radiators releasing excess body heat. Presumably this must be why the Irish elk became extinct — they had such large antlers that they froze to death.

The central part of the book on the developmental anatomy of antlers and a review of regeneration in mammals was very enlightening, and is the only section of any real interest to developmental biologists such as myself. Antlers are a remarkable example of mammalian regeneration and their initial histogenesis shows many of the cellular characteristics of amphibian limb regeneration. What is more, they can grow at the very high rate of 1cm per day. The last part of the book deals with systemic and environmental controls of antler development.

Overall, the book was a pleasure to read and it stimulated my interest in the subject. However, scientifically, I was left with a feeling of dissatisfaction. This is not a reflection on the author but upon the current problems of antler research. For example, there is a great deal of anecdotal and contradictory evidence, conclusions are often based on observations of a single animal, many experiments are uncontrolled and one has to wait at least a year for the results. With problems like that I can see why this is a neglected field of investigation. Although, as Goss says, the antlers of deer are very valuable to the developmental biologist, for those who do not wish to take on these problems I suggest reading his book instead. □

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Close up on spores — a scanning electron micrograph of three submature basidiospores of *Ganoderma applanatum* (x8,000). The illustration is taken from *Atlas of Airborne Fungal Spores in Europe*, edited by Siwert Nilsson and recently published by Springer-Verlag. Price is DM 128, \$55.20. This particular SEM was taken by J. Keller of the Université de Neuchâtel, Switzerland.

Biological Science

- BERLINER, I.J. and REUBEN, J. (eds). *Biological Magnetic Resonance*, Vol. 4. Pp. 340. ISBN 0-306-40968-2. (Plenum: 1982.) \$42.50.
- BERNS, M.W. *Cells*, 2nd Edn. Pp. 255. Pbk ISBN 0-03-061578-X. (Sauders College: 1983.) Np.
- BEWLEY, J.D. and BLACK, M. *Viability, Dormancy, and Environmental Control*, Vol. 2. *Physiology and Biochemistry of Seeds in Relation to Germination in Two Volumes*. Pp. 375. ISBN 3-540-11656-7/0-387-11656-7. (Springer-Verlag: 1982.) DM 128, \$51.20.
- BOYER, P.D. (ed.). *The Enzymes*, Vol. 15: *Nucleic Acids*, Part B. Pp. 659. ISBN 0-12-122715-4. (Academic: 1982.) \$79.50.
- BRAAK, E. *On the Structure of the Human Striate Area*. *Advances in Anatomy, Embryology and Cell Biology*, Vol. 77. Pp. 87. Pbk ISBN 3-540-11512-9/0-387-11512-9. (Springer-Verlag: 1982.) DM 52, \$23.10.
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- CARAFOLI, E. and SCARPA, A. (eds). *Transport ATPases*. *Annals of the New York Academy of Sciences*, Vol. 402. Pp. 604. Hbk ISBN 0-89766-196-6; pbk ISBN 0-89766-197-4. (New York Academy of Sciences: 1982.) Hbk np; pbk \$120.
- CARLSON, R. and GRANSTRÖM, B. (eds). *The Representation of Speech in the Peripheral Auditory System*. *Proceedings of the Symposium held May 1982*. Swden. Pp. 291. ISBN 0-444-80447-1. (Elsevier Biochemical: 1982.) \$68.75, Dfl. 148.
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- COUGHTREY, P.J., JACKSON, D. and THORNE, M.C. *Radionuclide Distribution and Transport in Terrestrial and Aquatic Ecosystems: A Critical Review of Data*, Vol. 3. Pp. 372. ISBN 90-6191-280-6. (Balkema, The Netherlands: 1983.) £30.
- COUGHTREY, P.J. and THORNE, M.C. *Radionuclide Distribution and Transport in Terrestrial and Aquatic Ecosystems: A Critical Review of Data*, Vol. 1 and Vol. 2. Vol. 1 pp. 496; Vol. 2 pp. 500. Vol. 1 ISBN 90-6191-278-4; Vol. 2 ISBN 90-6191-279-2. (A.A.

- Balkema, The Netherlands: 1983.) Vol. 1 £30; Vol. 2 £30.
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- CRUZ, H. *Creation and Evolution*. Pp. 81. No ISBN. (Catholic Publications Bureau, Colombo: 1982.) Rs 25.
- DALE, J.E. and MILTHORPE, F.L. (eds). *The Growth and Functioning of Leaves*. Pp. 540. ISBN 0-521-23761-0. (Cambridge University Press: 1983.) £50.
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- DOWBEN, R.M. and SHAY, J.W. (eds). *Cell and Muscle Motility*, Vol. 3. Pp. 311. ISBN 0-306-41157-1. (Plenum: 1983.) \$39.50.
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- EDWARDS, G. and WALKER, D.C. *Mechanisms, and Cellular and Environmental Regulation, of Photosynthesis*. Pp. 542. Hbk ISBN 0-632-00757-5; pbk ISBN 0-632-00767-2. (Blackwell Scientific Publications: 1983.) Hbk £32; pbk np.
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- Flora of Australia, Vol. 18: *Lecythidales to Batales*. Pp. 420. Hbk ISBN 0-644-02017-2; pbk ISBN 0-644-02018-0. (Bureau of Flora and Fauna: 1982.) Hbk \$A34; pbk \$29.
- FONG, F.K. (ed.). *Light Reaction Path of Photosynthesis*. *Molecular Biology, Biochemistry and Biophysics*, Vol. 35. Pp. 342. ISBN 3-540-111379-7/0-387-11379-7. (Springer-Verlag: 1982.) DM 138, \$55.20.
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- ROE, F.J.C. *Microbiological Standardisation of Laboratory Animals*. Pp. 116. ISBN 0-85312-556-2/0-470-27401-8. (Ellis Horwood/Halsted: 1983.) £16.50, \$33.15.
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- THOMPSON, R. and CASEY, R. (eds). *Perspectives for Peas and Lupins as Protein Crops*. *Proceedings of an International Symposium held October 1981, Italy*. Pp. 388. ISBN 90-247-2792-8. (Martinus Nijhoff: 1983.) Dfl. 125, \$54.50.
- WARD, Jr., A.A., PENRY, J.K. and PURPURA, D.P. (eds). *Epilepsy: Research Publications*. *Association for Research in Nervous and Mental Disease*, Vol. 61. Pp. 416. ISBN 0-89004-846-0. (Raven: 1983.) \$61.38.
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- WILLIAMSON, R. (ed.). *Genetic Engineering* 4. Pp. 20. ISBN 0-12-270304-9. (Academic: 1983.) £11.80, \$22.

Psychology

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- CHAPMAN, A.J. and GALE, A. *Psychology and People: A Tutorial Text*. Pp. 528. Hbk ISBN 0-333-33145-1; pbk ISBN 0-222-333-33147-8. (Macmillan Press, London: 1982.) Hbk £15; pbk £6.95.
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- SHEPHERD, M. and ZANGWILL, O.L. (eds). *Handbook of Psychiatry*, Vol. 1: *General Psychopathology*. Pp. 307. Hbk ISBN 0-521-23649-5; pbk ISBN 0-521-28137-7. (Cambridge University Press: 1983.) Hbk £27.50; pbk £9.95.
- TULVING, E. *Elements of Episodic Memory*. *Oxford Psychology Series*, No. 2. Pp. 351. ISBN 0-19-852102-2. (Oxford University Press: 1983.) £17.50.

History of Science

- CORSI, P. and WEINDLING, P. (eds). *Information Sources in the History of Science and Medicine*. Pp. 531. ISBN 0-408-10764-2. (Butterworth: 1983.) £30.
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- WEBSTER, C. *From Paracelsus to Newton: Magic and the Making of Modern Science*. Pp. 107. ISBN 0-521-24919-8. (Cambridge University Press: 1983.) £12.50.

Salon du Laboratoire

The companies featured in this selection will all be displaying at the Salon du Laboratoire exhibition to be held in Paris, France, 5-10 December

● J. Bibby Science Products has increased its reagent bottle range with the introduction of a new ISO-style bottle. The new ISO (International Standards Organization) Pyrex bottles in borosilicate glass are available in sizes from 50 ml up to 10 litres. Standard reagent bottles are available in sizes from 25 ml up to 20 litres. The ISO Pyrex reagent bottles are supplied with a light blue cap and a colourless pouring ring for use up to temperatures of 140°C. A heavy duty red cap and pouring ring are also available for use up to temperatures of 180°C.

Circle No. 100 on Reader Service Card.

● Sartorius is now introducing a new ultra-microbalance — the 4504 MP8. This unit offers an electronic weighing range of 120 mg to within $\pm 0.1 \mu\text{g}$. Dial-in weights take the total capacity up to 4.02 g. The 4504 MP8 features a remote motorized pan extractor, electronic tare, automatic calibration, standard RS 232 data output, self checking diagnostic sequencing and 'operator-set' operating parameters. The 4504 incorporates the MP8 microprocessor. It is designed for use in industrial analysis in research and development as well as in monitoring working conditions for airborne pollutants.

Circle No. 101 on Reader Service Card.

● The Buchler range of four constant flow peristaltic pumps is now available from Gallenkamp. All of the pumps feature Buchler's pumping disc and pressure bar design which allows a continuous length of tubing to be inserted even while the pump is running. Each disc forms a pumping channel with ten steel rollers around the perimeter, five of which are in constant contact with the tubing. This minimizes seepage and pulsation. The Buchler pump has a nylon ribbon between the rollers and tubing which reduces wear and smooths the flow. The mechanism of the pumps is enclosed in a solvent- and impact-resistant polypropylene casing. Separate switches are provided for forward or reverse pumping and selection of maximum speed for fast priming. The pumps have high-torque motors so that viscous solutions can be moved.

Circle No. 102 on Reader Service Card.

● A new range of laboratory water coolers and recirculators is being launched by F. and R. Cooling. This LCU series gives temperature control to 0.1°C. The instruments have a virtually noiseless water pump, a water filter, a fan-cooled condenser and a selection of refrigeration and electrical controls. Cooling capacities range from 1.5 kW per hour (1,250 kcal per hour) to 15 kW per hour (12,500 kcal per hour). This means that the coolers can be used with lasers, X-ray spectrometers, electron beam equipment, vacuum pumps and centrifuges.

Circle No. 103 on Reader Service Card.

● Cathodeon will be exhibiting its spectral sources at the Salon du Laboratoire show. The new items on display will include an increased range of multi-element hollow cathode lamps for all atomic absorption instruments. There will also be examples of deuterium lamps and a pulsed xenon arc lamp on show. These lamps are available with or without their associated power supplies.

Circle No. 104 on Reader Service Card.

● Leybold-Heraeus now has a range of analysers with the generic name Binos for monitoring gases and vapours. The analysers include those using IR, UV, visible, thermal conductivity and mass spectrometry.

Circle No. 105 on Reader Service Card.

● Two new magnetic stirrers, one with a hotplate (IKAMAG RH) and the other without (RO), have been introduced by Sartorius Instruments.

Circle No. 106 on Reader Service Card.

● Elga, in association with Filtro, will exhibit the new Elgastat MicroMeg laboratory deionizer at the Salon du Laboratoire exhibition. The MicroMeg can operate at flow rates of up to 90 litres per hour and will provide a treated water quality of 1-10 M Ω cm. The design of this deionizer incorporates a colour-coded conductivity meter. MicroMeg can be supplied with exchangeable resin cartridges which can be returned to either Elga's or Filtro's central facility for regeneration. Alternatively, disposable resin cartridges can be supplied. Elga will also display a range of laboratory deionizers and reverse osmosis equipment.

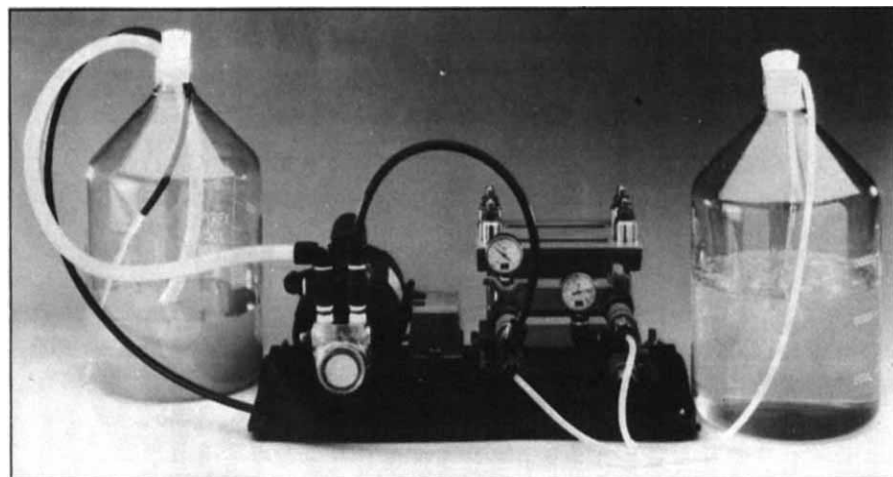
Circle No. 107 on Reader Service Card.

● There will be a number of Millipore's filtration systems on display at the Salon du Laboratoire exhibition. These include the Millititer system, which can filter 96 samples simultaneously. It consists of a microporous membrane which is soldered onto the bottom of a standard 96-well plate and a vacuum filtration support. Three Millititer plates are available, type HA, with a 0.45- μm nitrocellulose membrane, and type GV and type SV, both with Durapore membranes. The tangential filtration system will also be on display (see *Nature* 306, 302).

Circle No. 108 on Reader Service Card.

● An autosampler with variable injection volume is now available from Spectra-Physics. The SP8110 allows the injection of small volumes so that 1 μl can be injected from 5- μl samples. It can also be used to inject different volumes of a standard solution to give a multi-level calibration.

Circle No. 109 on Reader Service Card.



The tangential filtration system from Millipore

These notes are based on information provided by the manufacturers. For further details circle the appropriate numbers on the Reader Service Card bound inside the journal.

● A new laboratory automation system is now available from Philips. The Philips automated laboratory management system (PALM) is modular. It uses a mini-computer from the DEC PDP 11 family as the main processor, into which a choice of analytical instruments and input/output terminals can be connected. The computer can also be linked into a larger data processing complex by means of DEC's standard network software. It can also be connected on-line to a mainframe unit for centralized storage or automated plant control. The system's active data base can contain details of up to 4,000 current samples for which progress through the laboratory is automatically tracked. When any samples are awaiting operator attention, their presence is automatically signalled by the computer. Backlog reports can also be generated. Individual analysis results are automatically collated.

Circle No. 110 on Reader Service Card.

● Carlo Erba Strumentazione has introduced the Mega series of gas chromatographs. The Mega series allows a high level of dialogue between the operator and the instrument in the areas of parameter management and data processing. The system can operate unattended using either the HS 250 headspace sampler or the AS-V 570 automatic liquid injector. A choice of five detectors is available, including flame ionization, nitrogen/phosphorus, electron capture, flame photometric and thermal conductivity. Multi-detection operation is possible, using combinations of most of the above detectors.

Circle No. 111 on Reader Service Card.

● As a control for testing glycosylate haemoglobin assays Ortho Diagnostic Systems has introduced bi-level assayed glycohaemoglobin control. This contains two levels of HbA_{1c} aimed at the normal (I) and elevated (II) ranges. This control is prepared from whole blood and is assayed by microchromatography and electrophoretic methodologies. Ortho bi-level assayed glycohaemoglobin control is packaged in 1-ml vials (5 × 1 ml of each level). It is stable for up to 5 days at 2–8°C.

Circle No. 112 on Reader Service Card.

● J. Bibby Science Products has introduced a new water still which has an output of 4 litres per hour of single distilled water. The W14S still incorporates an automatic cutout that prevents overheating. An automatic control maintains the correct level of water in the boiler, irrespective of variations in mains pressure. The heating element is sheathed in Vycor, a 96% silica glass. The W14S can be mounted on a bench or wall and connects to normal mains water.

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● Tecator has introduced FIAstar, a fully automated flow injection analysis system. FIAstar is suitable for the analysis of agricultural, pharmaceutical and environmental substances. The system can carry out up to 300 analyses per hour, with response times of 5 s. The samples are introduced into an unsegmented stream after the pump, thereby reducing dispersion. The system is closed so that the risk of exposure to toxic reagents is reduced.

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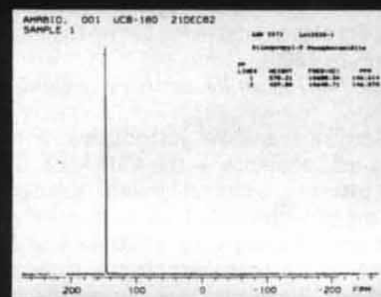
● Ortho Diagnostic Systems has extended its range of bovine-based controls with the introduction of unassayed control serum levels I, II and III (bovine). The levels are targeted in the low normal, high normal and elevated ranges. Electrolyte values in the low, mid and elevated ranges are provided in conjunction with three levels of cholesterol, triglyceride and direct bilirubin. There are also three levels of accompanying TRIS carbonate diluent, which provides elevated levels of CO₂ ranging from 15 mmol per litre in level I to 35 mmol per litre in level III.

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● A line of filter units for the sterile filtration of liquids or gases is now available from Millipore. Recent additions to the sterile filter unit line include the Millex-GV filter and the Sterivex-GV filter unit. The Millex-GV unit incorporates the new 0.2-μm hydrophilic polyvinylidene fluoride Durapore membrane. The Sterivex-GS filter unit is a pressure-driven device.

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Millipore's line of sterile filtration units.

Awards

The division of physiology, cellular and molecular biology of the US National Science Foundation is to award 20 two-year postdoctoral research fellowships in plant biology. The deadline for submitting applications is January 1984 and the fellowships will be announced in March 1984. Only United States nationals who have received a doctoral degree in a biological or a physical science more recently than 1 January 1980 or expect to earn their degree not later than 1 January 1985 are eligible. Their interests must lie within the fields of physiology, cellular and molecular biology. Applicants should have made arrangements for research and training with a scientific adviser at the institution where they intend to hold their fellowships. The fellowships provide a stipend, an institutional allowance and a special allowance to help meet the costs associated with research. It is paid at a rate of US \$1,800 per month. Further information is available from NSF Postdoctoral Research Fellowships in Plant Biology, PCM, Room 325, National Science Foundation, Washington DC 20550, USA.

The US Committee on Institutional Cooperation has set up a minorities fellowships programme in science, mathematics and engineering for 1984-85. The programme is designed to increase the representation of minority groups in all scientific fields. It is open to American Indians, black Americans, Mexican-Americans and Puerto Ricans who are US citizens and who hold, or will receive, a bachelor's degree from a regionally-accredited university or college by August 1984. The award provides financial support for four years, consisting of full tuition for the academic year plus an annual stipend of US \$6,000. The award will be renewed annually, contingent upon satisfactory performance and normal progress towards a PhD. Further information is available from CIC Minorities Fellowships Program, Kirkwood Hall 111, Indiana University, Bloomington, Indiana 47405, USA.

The International Union of Biochemistry (IUB) has set up a number of awards to enable young biochemists to attend the thirteenth IUB congress. The fellowships will support part of the cost of travel (up to half the listed economy fare) and subsistence during the congress. Preference will be given to residents of countries where the practice of biochemistry is still underdeveloped. Further information may be

obtained from Dr R. L. Hill, Department of Biochemistry, Duke University Medical Center, Durham, North Carolina 27710, USA.

Appointments

Professor R.J. Harrison, formerly professor of anatomy at the University of Cambridge, has been elected chairman of the trustees of the British Museum (Natural History) from 1 January 1984. He succeeds Professor T.R.E. Southwood, who is retiring.

Miscellaneous

The responsibilities of the directors of the British Science and Engineering Research Council have been changed. **Dr Harry Atkinson**, the director of science, is responsible for science, astronomy, space and nuclear physics. He is also responsible for the international section. **Mr Tony Eginton**, the director of engineering, is responsible for the engineering board and engineering directorates. **Mr John Visser's** duties as director of administration are unchanged.

Meetings

1-5 April, **European Conference on Surface Science**, York (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 8X, UK).

1-5 April, **AG ENG 84, an International Conference on Agricultural Engineering**, Cambridge, UK (Dr G.F. Forster, National Institute of Agricultural Engineering, Wrest Park, Silsoe, Bedfordshire MK45 4HS, UK).

2-6 April, **International Conference on Nuclear Methods in Environmental and Energy Research**, Mayaguez, Puerto Rico (Dr James Vogt, General Chairman, American Nuclear Society, University of Missouri, 214 Research Reactor, Columbia, Montana 65211, USA).

3-6 April, **Energy Options, The Role of Alternatives in the World Energy Scene**, London (Conference Services, IEE, Savoy Place, London WC2R 0BL, UK).

5-7 April, **International Conference on Infant Studies**, New York (Dr Bernard Karmel, ICIS Program Chair, Department of Pediatrics, Mount Sinai Medical Center, 1 Gustave Levy Place, New York 10029, USA).

9-11 April, **Faraday Discussion on Interfacial Kinetics in Solution**, Hull, UK (Mrs

Y.A. Fish, The Royal Society of Chemistry, Burlington House, London W1V 0BN, UK).

9-14 April, **International Symposium on Satellite Aided Search and Rescue**, Toulouse, France (Centre National d'Etudes Spatiales, Département des Affaires Universitaires, 18, avenue Edouard-Belin, 31055 Toulouse, France).

10-13 April, **Intermag '84, International Magnets Conference**, Hamburg, FRG (Professor Walter Proebster, Conference Chairman, IBM Deutschland GmbH, Research and Development, 7030 Böblingen, FRG).

15-18 May, **Symposium on Biotechnology for Fuels and Chemicals**, Gatlinburg, Tennessee, (Mr Charles Scott, Sixth Symposium on Biotechnology, Oak Ridge National Laboratory, PO Box X. Oak Ridge, Tennessee 37830, USA).

16-18 April, **World Conference on Inflammation, Antirheumatics, Analgesics and Immunomodulators**, Venice, Italy (Scientific Secretariat, First World Conference on Inflammation, Istituto di Farmacologia, Via Roma 55, 56100 Pisa, Italy).

17-19 April, **Annual Meeting of the European Society for Clinical Investigation**, Milan, Italy (Organizing Secretariat, Fondazione Giovanni Lorenzini, Via Monte Napoleone, 23-20121 Milan, Italy).

17-19 April, **International Symposium on Analytical Methods and Problems in Biotechnology**, Noordwijkerhout, The Netherlands (Miss Tiny Nijhof, Secretary of the Organizing Committee, c/o Elsevier Science Publishers, BV, PO Box 330, 1000 AH Amsterdam, The Netherlands).

17-19 April, **International Congress on Ozone and Biology**, Rennes, France (Mr Alain Laplanche, Symposium IOA, Ecole Nationale Supérieure de Chimie, avenue du General Leclerc, 35000 Rennes-Beaulieu, Rennes, France).

24-27 April, **European Congress of Biopharmaceuticals and Pharmacokinetics**, Salamanca, Spain (General Secretariat, Second Congrès Européen de Biopharmacie et Pharmacocinétique, Faculté de Pharmacie, PO Box 38, 28 place Henri Dunant, 63001 Clermont-Ferrand, Cedex, France).

29 April **EUCHEM Conference on Stereochemistry**, Bürgenstock, Switzerland (Professor L. Ghosez, Université de Louvain, Laboratoire de Chimie Organique de Synthèse, Place Louis Pasteur 1, B-1348 Louvain-La-Neuve, Belgium).

30 April-3 May, **Environmental Integration Technology Today for a Quality Tomorrow**, Orlando, Florida (Mr William Silver, Technical Program Co-chairman, Westinghouse Electric Corporation, PO Box 746, MS 504, Baltimore, Maryland, USA).

Seeking safe jobs in unsure times

*What qualities will employers be looking for in a "buyer's market" for labour, asks Richard Pearson**

WITH the new academic year now in full swing, the shrinking job market is worrying many final year students as well as those competing to enter higher education and pondering their choice of subject. The current ethos is that vocational subjects offer the best employment prospects. How then have science graduates fared so far in the 1980s and how can students and careers advisers assess which jobs and careers are likely to be in demand five or more years ahead? The "cycle of mismatch" caused by the short-term leads and lags attending market signals is the obvious problem (see *Nature*, 308, 83; 1983). The chief need when assessing job prospects is to understand the changing pattern of employers' demands and the critical features, economic, social and technical, likely to bring about future change. Indeed the only sure thing about a detailed long-term manpower forecast is that it will be wrong.

The United Kingdom is unique in having up-to-date information about the transition from higher education into employment. Since the 1960s, data have been collected from graduates about their "first destinations" six months after graduation. The published data provide us with a valuable time-perspective on changing graduate employment and on the jobs found by graduates in different subject groups.

What, then, were the employment experiences of those graduating in science in the early 1980s, and how have they been affected by the general rise in unemployment? Statistics can of course hide as much as they reveal and, while the precise levels of under-employment or of unemployment may be debated, the statistics do reveal the main trends. Graduate unemployment has grown sharply over the past five years (Fig. 1), and one in six of those graduating in 1982 were unemployed six months later. Science subjects have fared worse than the average over the years, while engineering and the professional disciplines such as medicine have fared best.

The differences between science subjects were, however, even greater, with 19.2 per cent of biologists being unemployed in 1982, worse than in chemistry (14.3 per cent) and a rate more than twice that for mathematics and computing science (9.3 per cent). By contrast, unemployment in

electrical/electronic engineering was down at 7 per cent (Table 1). Women in the sciences had a 12 per cent unemployment rate while the figure for their male counterparts was considerably higher at around 17 per cent.

Although unemployment was rising rapidly, the majority of science graduates still entered some form of employment (43 per cent) or further training (33 per cent), with most of those in employment working in industry. However, in terms of job content, only just over one in ten went into scientific or related work.

Table 1 Unemployment six months after graduation

Biology	19.2%
Chemistry	14.3%
Maths/computer science	9.3%
All science	16.5%
Electrical engineering	7.0%
All engineering	11.0%
All graduates	13.5%

Figures are for 1982. Source: *Universities Statistical Record*.

The majority of graduates have, therefore, gone into non-vocational jobs. What, then, has been the nature of these jobs and what qualities have recruiters been seeking? While job seekers usually expect jobs in multinational companies to be different from those in, say, a local food processing company, many do not realize that there are as many differences in jobs and careers within organizations as between them. There is no such thing as a "graduate" job. Employers seek to fill a diversity of jobs for many different

reasons.

Thus graduates may be sought for their scientific knowledge, or because they have a "trained" mind. Some are sought as high-fliers, others to fill short-term vacancies with prospects only to junior management.

Some recruits are expected to be productive straight away, others are carefully groomed for senior management. Different jobs need different types of people, a fact not always recognized by the recruiters themselves. It is a foolish employer who seeks to find the "best" graduates available when the jobs available offer only limited scope and career potential. It is only for the specialist functions, which account for perhaps one third of the vacancies, that degree subject is critical, and in the scientific area a postgraduate qualification is often a necessary entry requirement. For most jobs, including many of those in science, it is the personal qualities that are important.

Looking ahead to the late 1980s, the chief determinant of job prospects is obviously going to be the state of the economy. For the potential job seeker, it will be important to remember that strictly vocational jobs will continue still to be in a minority, and that only in certain areas, probably related to information technology, is it likely that all those qualifying in technical subjects will be able to move into related specialist jobs. The prime need will be for flexibility about type of job, employer, and subsequent use of subject. Most importantly, personal qualities — motivation, communication skills and especially numeracy — will continue to be as critical in the future as in the past. □

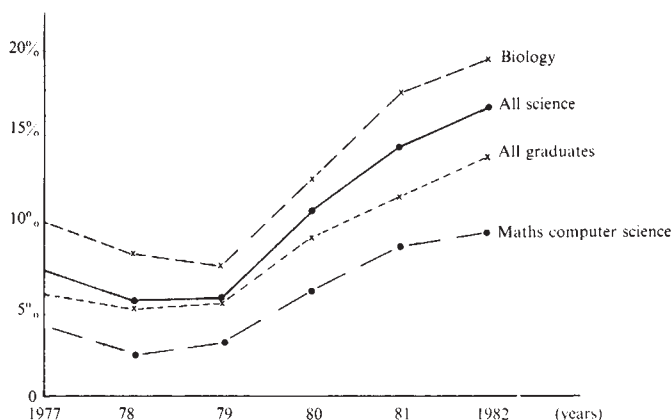


Fig. 1 Unemployment among UK university graduates six months after graduation for the years 1977–82.

*Institute of Manpower Services, University of Sussex, Falmer, Brighton BN1 9RF, UK.